

**The Effect of Consumption of
Australian Combat Rations on
Military Personnel after a
Medium-Term Field Exercise**

Christine Booth, Ross Coad,
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*Christine Booth, Ross Coad, Chris Forbes-Ewan, Gary Thomson and Philip Niro**

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ABSTRACT

The effect of combat ration pack (CRP) feeding on military performance in a tropical environment was assessed. Three groups received different diets: freshly prepared foods (Fresh group), a Full CRP and a Half CRP during the conduct of a routine training exercise over 12 days. Physical, cognition, immune and nutritional status were recorded. Freshly prepared foods were better consumed than CRP foods. A high rate of CRP item discards resulted in subjects eating insufficient food for their energy and carbohydrate requirements and hence significant weight loss, protein catabolism and immune suppression were observed for the two CRP groups. All subjects experienced poor sleep quality with no effect of dietary treatment. Subjects eating CRP reported greater levels of fatigue and negative emotions than the Fresh group. All subjects had poor folate and vitamin K status, which tended to become worse during the exercise period. Subjects drank insufficient water to prevent dehydration and a high rate of cigarette smoking contributed to poor antioxidant status. Despite these negative effects, cognition and physical fitness were maintained over the course of the exercise.

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Executive Summary

There is rising international defence concern regarding the safety of long term CRP usage. The major outcome of three years of deliberation by Action Group 16 of Group Human Resources and Performance of The Technical Cooperation Program (TTCP) is an agreement that Australia will be the lead nation for a series of detailed nutrition studies.

Australian and international field evaluations of the use of combat ration packs (CRP) have been reviewed [3]. That review, which provided the background rationale for the present study, highlighted that few international studies and even fewer Australian studies have documented the effects of long-term CRP usage. In fact, there have been no evaluations of the effects of long term consumption of Australian CRP since the 1960s. A common feature of long-term CRP usage is weight loss. In most cases these weight losses are believed to be tolerable. However, some US evaluation studies revealed measurable decrease in muscle strength and immune competence as well as weight loss after one month of CRP consumption as sole nutrition.

This study aimed to document the effect of medium-term CRP feeding on military performance in a tropical environment. The study was conducted during Exercise Northern Awakening, RAAF Base Scherger. Airfield Defence Guards (ADGs) of the Second Airfield Defence Squadron (2AFDS) were recruited into Group 1 (n=10, Full CRP, 15000 kJ/day), Group 2 (n=10, Half-CRP, 7500 kJ/day) and Group 3 (n=13, Fresh group, fresh rations, 15000 kJ/day). Nutritional requirements were estimated by use of the Ration Expert Advisor Program (REAP™). The nutrition study was conducted during a routine ground defence training exercise over 12 days. Indices of physical fitness, cognition, immunocompetence and nutritional status were recorded. Energy expenditure of selected subjects was measured by the doubly-labelled water method. Activity and sleep quality were recorded by use of wrist Actigraphs™. Dietary intake was recorded.

Recorded activity and calculated energy expenditure indicated similar levels of physical activity for the three treatment groups. All subjects experienced a highly disruptive and poor sleep quality with no apparent effect of dietary treatment. There was no change in cognition over the study period and no differences were found between the dietary treatment groups.

Conclusions and Recommendations

1. The menu, which was designed by use of REAP™, was found to maintain the nutrition status of subjects in the Fresh group.
 - Further work to tailor REAP™ to Australian conditions is warranted.
2. Freshly prepared foods were better consumed than CRP foods. A high rate of ration item discards by ADGs being fed CRP as their sole nutrition resulted in these subjects eating insufficient food for their energy and carbohydrate needs.
 - Future CRP acceptability studies should focus on understanding the reasons why soldiers discard up to one quarter of the items in the CRP pack.
3. The effects of inadequate sleep and high physical demand under the hot humid conditions of this training exercise coupled with food deprivation were not sufficient in the medium-term (12 days) to result in a measurable loss of physical fitness or cognition. Furthermore there was metabolic and psychological evidence that subjects eating CRP were able to adapt to a restricted dietary intake, because there were no differences in metabolic, physical or psychological measures between the two CRP groups.
4. Restricted food consumption by the subjects in the CRP groups resulted in mild symptoms of weight loss, suppressed immune function, loss of visceral protein, increased fatigue, loss of vigour, and increased feelings of confusion. Carbohydrate intake was negatively associated with run times during the fitness testing.
 - Further research is required to evaluate the potential of alternate CRP items or supplements, which may reverse adverse metabolic and psychological effects and thereby optimise Deployed Forces health and performance.
 - The present study was of medium duration (12 days). A similar CRP study over 30 days is required to determine the effect of ration pack feeding during sustained operations. Such a study should also monitor the subjects during the recovery phase after completion of the training exercise.
5. Vitamin status of the ADGs as determined by measurement of homocysteine, vitamin K and total antioxidant capacity was poor before Exercise Northern Awakening and tended to be worse on completion of the exercise.
 - The finding of poor micronutrient status amongst the ADGs lends support to the need for a comprehensive nutrition education program for ADF personnel.
 - The findings are important and require further investigation, because poor vitamin K, folic acid and antioxidant status are indicators of increased cardio-vascular disease risk.
6. There was some evidence that smoking suppressed immune system function and exacerbated weight loss.
 - Although not conclusive, cigarette smoking was implicated in decreased military performance. A detailed study of the effects of smoking during training exercises may be warranted.
7. Serum IGF-1 and salivary sIgA:Alb were sensitive biomarkers of dietary energy deficit. These biomarkers will be useful in future nutrition studies.

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Christine graduated from the University of Queensland (UQ) with BSc(Hons) and PhD (1992) in biochemistry. She has also obtained qualifications in education (Dip Ed, UQ) and dietetics (Grad Dip Nutr Diet, QUT). She has membership of the Australasian Association of Clinical Biochemists, American Association of Clinical Chemists, Dietitians' Association of Australia, Nutrition Society of Australia (Secretary Tas branch), the Australian Institute of Food Science and Technology, Tasmania's Food Advisory Council and is an Honorary Research Associate in the School of Human Life Sciences, University of Tasmania. Christine has held research positions within UQ and QUT and a supervising scientist position within Chemical Pathology at Royal Brisbane Hospital. In her four-years employment as senior chemist at the Defence Nutrition Research Centre Christine has been investigating the nutritional status of soldiers and the effects of long-term combat rationing on health and military performance.

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1. Introduction

Generally, soldiers can expect to be fed with combat ration packs (CRP) only until field kitchens are established and fresh feeding can commence. The principle of rationing in the field in both peace and war is that soldiers should be fed to the best possible standard, using fresh foods wherever possible [1]. However, recent experience has shown that CRP are often the only practical means of feeding for extended periods. For example, during the initial Operation Warden deployment (in East Timor) ADF members of INTERFET were rationed for up to seven weeks with Combat Ration One Man with little or no supplemental fresh foods (Captain Steve Prigg, office of DGHS, *pers. comm.*). Hygiene of food preparation, storage and transport prevented the early establishment of field kitchens (WGCDR James Ross, SO1 HD and SO1 HHPR, office of DGDHS, *pers. comm.*). Such prolonged use of CRP is not in accordance with the recommendations of SUPMAN 4 [1], which suggests that the Combat Ration One Man should be used for a maximum of 16 days during peace and 20 days during war. However, SUPMAN 4 also suggests that during times of emergency CRP are suitable for rationing as long 'as necessary'. Hence, Australian combat rations are intended to sustain soldiers over the long term.

The ADF uses three major types of CRP – Combat Ration One Man (CR1M), Patrol Ration One Man (PR1M) and Combat Ration Five Man (CR5M). The rationale behind the contents and use of each CRP is described in SUPMAN 4 [1]. A discussion of the nutritional adequacy and acceptability of Australian CRP is provided by Forbes-Ewan[2]. This document draws on surveys and chemical analyses conducted by DNRC.

The assumption that because a ration is nutritionally adequate (as assessed by theoretical means) it will sustain the soldier indefinitely is tenuous. This assumption implies that the soldier will eat the entire CRP even under adverse conditions and that theory has accounted for the metabolic demands of extreme environment, arduous exercise and the psychological stress of combat. The nutritional adequacy of CRP can be confirmed only by testing under realistic field conditions.

An extensive review of Australian and international field evaluations of combat rations, is provided by Forbes-Ewan and Waters [3]. This review highlights that few international studies and even fewer Australian studies have documented the effects of long-term ration pack usage. In fact there have been no long-term field studies of the effects on operational performance of Australian Ration Packs since the 1960's [4, 5].

Published scientific studies of CRP usage have extended over 14 to 34 days. One study monitored the nutritional status and immune function of soldiers during the Ranger Training Course [6]. Another series of studies investigated cold-weather field feeding [7]. Studies have included the effects of rationing with reduced energy and higher energy varieties; Meals Ready-To-Eat; Ration Light Weight - 30 days; and mixtures of

ration packs and fresh foods[6, 7, 8, 9, 10, 11]. In some cases, personnel consuming fresh foods have been used as control subjects for the studies. Most studies were uncontrolled.

The numbers of subjects enrolled in most studies have been small ($n < 15$ per group). In itself this would not have been a problem if repeat studies had been conducted. However, in most studies the data represents one-off studies involving only few subjects. Only one study used sample sizes that are large enough to permit reliable comparisons to be made [11].

The researchers have used the following measures as markers of the effect of long-term CRP usage:

- body composition (weight-loss, loss of fat-tissue, loss of muscle tissue);
- nutritional status (blood and urine chemistry);
- physical fitness (VO_2 max, anaerobic capacity), and
- mental performance (tests of mental agility and motor skills)

Although it is difficult to compare the published studies a few consistencies are apparent. All studies report weight loss as a feature of long-term CRP usage. However, in most cases these weight losses were believed to be tolerable, as evidenced by good hydration status and blood and urine chemistry. No overt vitamin or mineral deficiencies were reported. Weight loss varied between less than 1% after 10 days, 6% after a 34 day-study [11] and 15.6% (range: 9 - 23%) after the Ranger Training Course [8].

Despite no obvious disorder of blood or urine chemistry, in the 34-day study the weight loss was found to adversely affect physical performance. There was decreased isokinetic strength and VO_{2max} among subjects consuming RLW-30 when compared with control subjects consuming MRE. In the case of the Ranger studies loss of muscle protein resulted in a 23% decrease in lifting strength and a dramatic suppression in cellular immune status coinciding with increased rate of infection.

To address the dearth of data concerning the adequacy of Australian CRP field studies of CRP have been initiated.

There is rising international defence concern regarding the safety of long term CRP usage. The major outcome of three years of deliberation by Action Group 16 of Group Human Resources and Performance of The Technical Cooperation Program (TTCP), was an agreement that Australia would be the lead nation for a series of detailed nutrition studies. A nutritional study was therefore conducted in conjunction with Exercise Northern Awakening, a RAAF exercise conducted at RAAF Base Scherger during April 1999.

The aims of the RAAF exercise included the provision of training in Base Control Centre procedures, provision of training in short-term move execution for support units and provision of training for 2-AFDS in defence of the Air Field. The aim of the present study was to evaluate the nutritional adequacy of Australian CRP as sole source of nutrition over 12 days by use of dietary, biochemical, physiological and psychological means. In an effort to control for non-nutritional factors, the subjects enrolled in the study received one of three different dietary treatments - meals prepared from fresh foods (Fresh group) or CRP (full and half menus).

2. Methods and Materials

2.1 Subjects and study design

Thirty three ADGs of 2-AFDS RAAF Base Amberley were invited to take part in a nutrition study during Exercise Northern Awakening. Two ADGs in the Fresh group failed to complete the study due to injury and personal issues. The average age of the subjects was 22 years with a range of 18 to 32 years. The ADGs operated in three rifle flights (corresponding to infantry groups) and each group received a different dietary treatment; either:

Full CRP menu, 15000 kJ, n = 10,
Half CRP menu, 7500 kJ, n = 10, or
Freshly prepared meals, 15000 kJ, n = 13 (Fresh group).

Allocation of rifle flights to treatment group was random. This was the only practical way to conduct the experiment, because a random allocation of diets within rifle flights would have interfered with the conduct of the training exercise. The subjects were not informed ahead of time that they were included in the Full CRP, Half CRP or Fresh groups. In order to make the Half CRP ration more like the Full CRP, main meal items were selected to be low in energy density and some were re-packaged so that these subjects could still receive two main meals per day. Each menu was designed to provide similar breakdown of macronutrients as a percentage of total energy: 54% carbohydrate, 30% fat and 16% protein. Full CRP and fresh foods menus were based on nutritional requirements estimated by the Ration Expert Advisor Program (REAP™, US Army Systems, Natick) to provide adequate nutrition for the exercise.

The experimental procedures were approved by the Australian Defence Medical Ethics Committee (ADMEC protocol 134/98). Written consent was obtained from each participant after the details were explained. Copies of the information and consent forms are included in Appendix A.

2.2 Weather Conditions

Weather measurements were not taken by the scientific team. Measurements taken by the Bureau of Meteorology at the Weipa Aerodrome indicated that the temperature ranged between 24 and 33°C with an average of 33°C at 15:00 hours during the study period. Humidity ranged between 71% and 96% with an average of 73% at 15:00. Rain fell daily with the highest rainfall recorded being 100 mm, which fell within two hours at the Maintenance Camp, where the scientists were accommodated.

2.3 Presentations for medical attention

ADMEC approval was not sought for access to subjects' personal medical files. However, records of the total number of presentations for various conditions against dietary treatment group were maintained. The medical conditions included upper-respiratory tract infection (URTI), skin problems, joint or muscle problems, injury, dehydration, insect bites, exhaustion and "other conditions".

2.4 Dietary Treatments

Daily macronutrient requirements for the exercise were estimated by use of REAP™. This is a software program under development by USA Army Systems Natick, which requires input of expected weather conditions and operational information such as load carriage, terrain and activities to predict average energy, macronutrient and water requirements. The program translates this information into the suitable US rationing system and quantities for the military operation.

In an effort to prevent under-consumption of ration pack items, the two groups receiving CRP were provided with a combat ration menu. This allowed subjects to select their favourite items from a choice of main meals, starch foods, dairy foods, fruit-based foods, sugar-based foods, biscuits, muesli bars, energy bars and beverage powders. Items were sourced from CR5M, CR1M and PR1M packs. It was hoped that the novelty and choice would encourage subjects to eat all their CRP. Three-day CRP were individually packed for each subject. In addition to the ADF ration items two experimental high carbohydrate items, Ergo drink™ and Hooah! bar™, produced by US Army Systems, Natick USA were included as sugar and energy bar choices, respectively. The Full CRP menu provided an average of 15100 kJ, 126 g protein, 127 g fat and 500 g carbohydrate and the Half CRP menu provided an average of 7600 kJ, 64 g protein, 55 g fat and 250 g carbohydrate. A copy of the CRP menus is included in Appendix B.

The Fresh group received meals prepared from fresh foods and a ration supplement (brew kit). A copy of this menu is included in Appendix C. Breakfast, Lunch and Dinner meals provided an average of 12000 kJ and the brew kit provided a maximum of 4000 kJ (including 85 g carbohydrate). The menu provided an average of 15100 kJ, 210 g protein, 95 g fat and 510 g carbohydrate. The Fresh group were not given a menu

choice. The brew kit was issued as a three-day pack and meals were delivered to subjects in the field within one hour of preparation. Because of the adverse effect of chicken meat on the urinary excretion of 1-methylhistidine, a marker of dietary meat intake used in this study, chicken was excluded from the three menus.

2.5 Dietary intake measurement

CRP were issued as individually labelled packages with sufficient food for three days. Fresh subjects received individual meals labelled with their names. Subjects returned their food packaging and uneaten items within name-labelled containers/bags provided. The types and quantities of foods delivered to each subject were recorded and dietary intake was estimated by recording food discards and emptied food packaging.

Nutrient intake by the Fresh group was calculated using the DIET/1 NUTRIENT calculation software (Xyris Software, Brisbane, Australia), which used the NUTTAB 92 database, a database of Australian foods. Nutrient intake by the groups receiving CRP was calculated by use of an in-house food-composition database (ADF-Nut), which contains the macronutrient (protein, fat, carbohydrate, energy) composition for Australian CRP items analysed within our laboratory.

2.6 Physiological Measurements

2.6.1 Fitness Tests

A field test of aerobic power and upper body strength was conducted at RAAF Base Scherger at 16:30 hours on the day of arrival at the base and again at 16:30 hours on the last day of the study. The testing was supervised by a qualified RAAF Physical Training Instructor. Aerobic tests consisted of running 2.4 km in the subject's best time and upper body strength was tested by recording the maximum number of correctly performed sit-ups completed according to a cadence (maximum of 120) and recording chin-ups to volitional exhaustion. Subjects were familiar with these tests because they are commonly conducted as part of the Australian Defence Force Basic Fitness Assessment (BFA).

2.6.2 Hand-Grip Strength

Hand-grip strength was determined using a hand-grip dynamometer (Jamar Hydraulic Hand Dynamometer). The subject had two attempts with each hand to exert maximal force on the dynamometer. The higher result was recorded as 'hand-grip strength' for each hand.

2.6.3 Body Composition Measurements

The methods for recording anthropometric data were based on those of the International Society for the Advancement of Kinanthropometry (ISAK) [12]. Stature, weight, skinfolds, mid-upper arm circumference (MUAC) and bioelectric impedance measurements were recorded on the first and last days of the study.

The same experimenter performed the same measurements pre- and post-study on each subject. This eliminated the possibility of inter-operator differences affecting the results. All tests were conducted on the right side of the body (for those measurements that involved a choice of site).

2.6.3.1 Stature

The method of 'stretch height' was used to determine stature [12]. This requires that two experimenters be present—a recorder and an assistant. The subjects were required to stand barefoot with back to the wall, head directly under the head board of a wall-mounted stadiometer, heels touching the wall, feet close together. The subject's head was held in the 'Frankfort plane' (the orbitale is at the same height as the tragon) by the assistant. To achieve this the measurer cupped both hands under the jaw of the subject, with fingers reaching to the mastoid process on each side of the subject's head. The subject was instructed to breathe in deeply, stretch up (with feet remaining flat on the floor) and the assistant contributed to the stretch by gently lifting the subject's head, keeping the head in the Frankfort plane. With the subject holding his breath, the recorder lowered the head board firmly on to the subject's head, pushing his hair down. The subject stepped away and his stature was recorded.

2.6.3.2 Body weight

The subjects wearing only briefs were weighed on platform scales (Wedderburn model TI BWB700) to the nearest 50 g.

2.6.3.3 Skinfold thickness

Skinfolds were measured at four sites - biceps, triceps, subscapula, supraspinale by use of Harpenden Skinfold Calipers (British Indicator Ltd, Herts, UK) and percentage body fat was estimated by the method of Durnin and Womersley [13].

The sites had been marked with a fine-point felt-tip pen (the subject was 'landmarked'). Briefly, four anatomical landmarks were used to determine the sites for measurement: acromiale, radiale, iliocristale and iliospinale.

The skinfold was picked up with the thumb and forefinger at the marked site, with the back of the measurer's hand facing the measurer. The skinfold calipers were applied so that the calipers were about one cm from the fingers. The skinfold thickness was recorded two seconds after the calipers were applied. For the triceps and biceps, the skinfold is vertical; the calipers were applied about one cm inferiorly to the fingers. For the subscapula, the skinfold is at about 45 degrees to the vertical, so that the fold forms

a ridge that runs inferiorly and laterally. The measurement was taken about one cm laterally to the fingers. For the supraspinale, the skinfold runs posteriorly and medially at about 45 degrees. The measurement was made about one cm medially to the position of the fingers.

2.6.3.4 *Mid Upper Arm Circumference (MUAC)*

Following measurement of skinfold thicknesses, MUAC was determined with a tape measure as the circumference of the upper arm at the level of the mid acromiale-radiale (the site of the biceps/triceps landmark) of the right arm. Combining the MUAC with the triceps skinfold (TSF) measurement enables indirect determination of the arm muscle area (AMA). The following equations rely on the assumptions that the arm is circular in shape and that the skinfold calipers measure a double thickness of skin and fat [14].

$$\text{Bone free upper arm muscle area (mm}^2\text{)} = [(MUAC - \pi TSF)^2 / 4\pi] - 10$$

Bone free muscle area provides a good indication of the lean body mass and thus the skeletal protein reserves [15].

2.6.3.5 *Bioelectric impedance*

Bioelectric impedance was measured by use of the Seac BIM 4 bioelectric impedance analyser (BIA) (Seac Pty Ltd and Uniquet Ltd, QLD, Australia). The instrument uses a single electric frequency of 50kHz and uses the Lukaski prediction equation to estimate percentage body fat from a calculated estimate of TBW [16].

The bioimpedance method is based on the 'two compartment' theory of body composition: that the body can be divided into 'fat mass' (FM) and 'fat free mass' (FFM). Water and electrolytes (which conduct electricity) are found only in the FFM. Hence the extent of impedance of an electric current will depend on the relative proportions of FM and FFM in the body. A very small current strength (~200 μ A) is passed through the body and the resulting impedance is used to estimate FM and FFM.

Subjects were tested in a well-hydrated post-prandial state. Subjects were encouraged to have a large drink of water at least an hour before measurement with no further drink or food within one hour of measurement. The subject lay face-up with his legs slightly apart and hands resting next to his body, palms down. Care was taken to ensure that his hands were not touching any part of his body. His inner thighs were not permitted to be in 'skin-to-skin' contact. The subject had removed his right shoe and sock. Electrodes connected to the BIA were placed on his right hand and right foot as described in the manufacturer's instruction manual.

2.6.3.6 *Isotope dilution method*

Fat, by definition contains no water, so all body water is in the fat-free body compartment. Examination of human cadavers has shown the average fat-free water

content to be 72.4% (range 67.4% to 77.4%). Hence total body fat F can be estimated from the following equation from TBW [17]:

$$F \text{ (kg)} = \text{body weight (kg)} - \text{TBW (kg)} / 0.724$$

Total body water (TBW) was also estimated by observing the dilution of an accurate dose of water labelled with deuterium (0.1g per kg body weight).

Subjects provided a urine sample at RAAF Base Amberley to allow determination of baseline isotope levels. On the evening of day 1 and again on the last evening of the study each subject took their dose of labelled water. Because the first dose was taken within several hours of arrival, it was before a significant change in baseline isotope levels, from those measured in the pre-dose urine samples, could have occurred. The dose was administered mixed with tap water to make the volume about 300 mL. The subjects were requested to rinse the container with another 200 mL of tap water and to drink this. Subjects were informed of the importance of taking all the labelled water dose.

Subjects were provided with screw-top urine sample containers and requested to provide an early morning post-dose sample, in the form of a second (or later voiding) following administration of the DLW. Each day thereafter, when the tactical situation allowed, subjects provided another urine sample. The date and time of collection was recorded to the nearest five minutes. One of the scientific team (who had not taken labelled water) collected a daily urine sample so that the isotopic background could be monitored.

Deuterium analysis was performed by the School of Human Movement Studies at the Queensland University of Technology. Water was distilled from the urine samples before generation of hydrogen gas and measurement of deuterium by mass spectrometry.

2.6.4 Total Energy Expenditure (TEE)

All subjects were informed that they could be taking isotopically-labelled water and that their urine samples could be used for measurement of body composition and energy expenditure. Four subjects from each of group 1 (Full CRP) and group 2 (Half CRP) were selected for the TEE cohort to represent the range of body weights. These subjects were not aware that they had been selected for the DLW dose.

A range of doses had been prepared previously (weighed to 2 decimal places). Isotope dosage should depend on total body water (TBW). With no estimate of TBW available at the time of dosing, body weight was used to determine dosage. The labelled water given to these subjects contained oxygen-18 (0.25 g per kg body weight) and deuterium (0.1 g per kg body weight).

Each member of the cohort took their dose of DLW upon arrival at RAAF Base Scherger and urine samples were collected as described above.

The DLW method, which was used to estimate total energy expenditure, is based on the principle that deuterium is only lost from the body as water while oxygen-18 is lost in water and expired carbon dioxide (CO₂) [18]. Therefore the elimination rate of deuterium is proportional to body water turnover and the elimination rate of oxygen-18 is proportional to the sum of water turnover and CO₂ production. The difference between the elimination rates of the two isotopes is proportional to the rate of production of CO₂.

To convert proportional CO₂ production into absolute, an estimate is needed of total body water (TBW) and the mean respiratory quotient of the subject (obtainable from body composition changes and food quotients). TBW can be estimated by isotopic dilution (see above). Because TBW is likely to alter over a twelve-day period involving vigorous physical activity in the heat, two estimates were made (at the start and end of the study period) and the mean value was used in TEE calculations.

Oxygen-18 analysis by mass spectrometry was performed by the School of Human Movement Studies at the Queensland University of Technology. Dr Peter Davies performed the calculations necessary to estimate TEE.

2.7 Delayed-Type Hypersensitivity test

When healthy persons are re-exposed to recall antigens administered intradermally, an immune response is stimulated. This is typically in the form of a delayed-type-hypersensitivity (DTH) response with an area of induration and erythema occurring after 48 hours. DTH skin-test reaction is an *in vivo* test for immune competence and function of T-lymphocytes and macrophages. The Multi-test Cell Mediated Immunity (CMI) Delayed Hypersensitivity Skin test kit (CSL Biosciences), which uses doses of 7 antigens, is the most commonly used kit and was used for this study. Two readings were recorded in accordance with the manufacturer's instructions, 48 hours apart.

2.8 Activity measurements

Motionlogger Actigraphs, model BMA-32 (Precision Control Devices, Ft. Walton Beach, FL USA) were employed to assess patterns of rest and activity, total physical activity and to estimate duration and fragmentation of sleep. These monitors have been used in previous military and laboratory studies to provide information on individual and group physical activity levels and patterns of activity and sleep [19, 20]. The devices are 4 cm L x 3.1 cm W x 1 cm H, weigh 57 g and were worn on the wrist of the non-preferred hand using a standard wristwatch band. Each device contains a microcomputer, 32K of memory, an analog-to-digital converter and a piezoelectric sensor. They are powered by standard wristwatch batteries and can record continuously for up to 21 days. Twenty two subjects from the Full CRP group (n = 8),

Half CRP group ($n = 8$) and Fresh group ($n = 6$) wore the Actigraphs for the duration of the patrol exercise (ten days). Due to insufficient data being recorded three of the data sets from the Fresh group could not be used in the data analysis.

Data collected by the BMA-32 monitors were downloaded to a laptop computer for further analyses using the Action 3 software program (Ambulatory Monitoring, Inc.; Ardsley, NY). The monitors were programmed to sample total activity counts in one-minute blocks using a calibrated zero-crossing mode. These data files were scored for a sleep/wake state based on a pre-programmed, empirically derived sleep scoring algorithm. For the purposes of interpretation and analysis, the data is presented as 24-hour noon-to-noon intervals for the ten days of the study. Figure 1 presents a printout of a typical Actigraph™ profile.

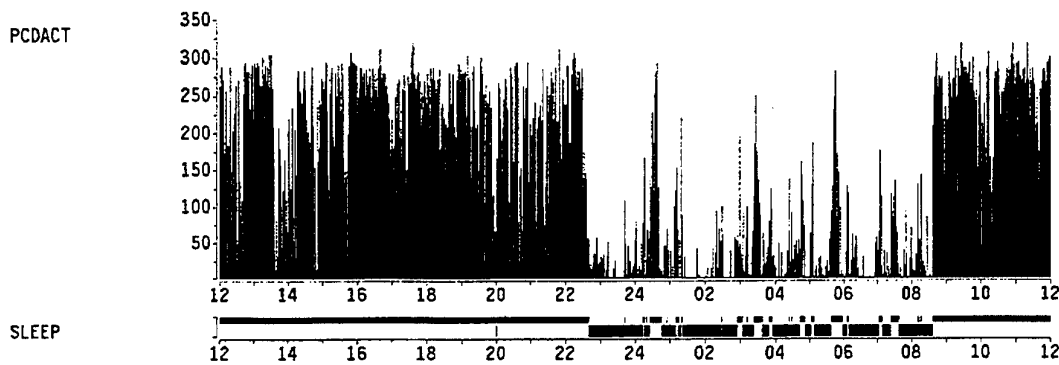


Figure 1: Actigraph Mini Motionlogger BMA-32 Monitor. Each vertical line plotted on the x-axis represents the summed total amount of movement exhibited by the wearer in a 1-minute period of time. Each individual plot represents a 24-hour period starting at 1200 hours on a specified date. Below each plot is estimated sleeping versus waking time. The thicker, lower bar nearer to the x-axis indicates when the subject's activity is indicative of sleep. The estimates of sleeping versus waking are generated automatically by the Action3 software using a pre-programmed, empirically derived sleep scoring algorithm. The subject in this example registered sleep from approximately 22:30 hours until approximately 08:30 hours the next morning. However, the number of awakening episodes within the sleep event would suggest a highly disruptive and fragmented sleep.

2.9 Biochemical Measurements

2.9.1 Urine analysis

Daily urine samples were collected from each subject at between 14:00 hours and 15:00 hours and were transported cold to the field laboratory. Samples were used for determination of hydration status by specific gravity measurement, determination of cigarette smoking by measurement of total nicotine metabolites, determination of muscle protein breakdown by measurement of 3-methylhistidine:1-methylhistidine ratio and creatinine, and measurement of stable isotopes. After specific gravity measurements the samples were stored frozen. Frozen samples were air-transported to the analytical laboratories.

Specific gravity was measured by use of a hand-held refractometer (Uricon-NE Specific Gravity Urine Specific Gravity Refractometer, Atago Co. Ltd, Australia). A range of 1.003 to 1.03 was used to indicate good hydration [21]. Total nicotine metabolites were determined using a colorimetric assay adapted for an automatic chemistry analyser (Cobass Bio, Roche, Australia) [22]. Urinary methyl histidines were determined by a high performance liquid chromatography (HPLC) assay which was adapted from the method of Simons and Johnson [23]. The assay uses reversed phase ion-pairing chromatography with o-phthalaldehyde post-column derivatisation and fluorescence detection.

2.9.2 Blood analysis

Subjects fasted overnight. The next morning before breakfast, a venous blood sample was drawn into a 10 mL tube containing lithium heparin, a 10 mL plain tube containing clotting activator and a serum separator and a 5 mL tube containing sodium EDTA. Samples were immediately placed in refrigerator at 4 °C. Within one hour of collection, the plasma and buffy coat were removed. The separated RBCs and plasma were stored frozen then air-transported frozen to the laboratory.

Total plasma homocysteine (Hcys) was defined as the sum of all homocysteine species in plasma, including homocysteine, homocystine, mixed disulfides, and protein-bound forms. All these were converted to Hcys by reduction with sodium borohydride then measurement by HPLC with fluorescence detection using a method adapted from Allena et al. [24]. Ferritin and C-Reactive Protein were measured by particle-enhanced nephelometric assay (Behring BNA) using manufacturer-supplied reagents. Human IL2 and IL6 were measured by competitive enzyme immunoassay (Accucyte kits, Cytimmune Sciences Inc, Maryland USA). IL2r was measured by endpoint enzyme immunometric assay (Milenia kit, Bio MediQ DPC Pty Ltd, VIC Australia). Insulin-like growth factor 1 (IGF-1) was measured by enzyme-linked immunosorbent assay using a non-extraction procedure (Diagnostic Systems Laboratories, NSW Australia). De-carboxy Prothrombin or PIVKA-II (Protein Induced in Vitamin K Absence – factor II)

was measured by enzyme-linked immunosorbent assay (Asserachrom PIVKA-II, Diagnostica Stago, France). Total antioxidant capacity (TAOC) was measured by incubation of plasma with 2,2'-azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS®) in the presence of a peroxidase and hydrogen peroxide to produce the radical cation ABTS®⁺. This colorimetric assay used reagents supplied by Randox Laboratories, UK.

2.9.3 Saliva analysis

Saliva samples were collected from each subject on five days (1, 3, 6, 9, 12). Subjects were asked to have a clean freshly rinsed mouth and to leave a cotton swab in their mouth without chewing until they had to swallow. The cotton swab was collected into a Salivette tube (Starstedt, SA Australia). Samples were transported cold to the field laboratory where they were centrifuged then the saliva stored frozen. Albumin and IgA in saliva were measured by nephelometric assay (Behring BNA) using manufacturer-supplied reagents (antisera to Human IgA α chain and human albumin).

2.10 Psychological measurements

2.10.1 Cognitive testing

A battery of tests was selected that would, given limitations in testing time due to the operational requirements of the field exercise, provide information on a variety of key cognitive parameters. The tests employed assessed relatively basic functions like reaction time and vigilance, as well as more complex functions such as attention, memory and reasoning. All cognitive tasks were administered on Panasonic CF-V21P 486 laptop computers at the start of the exercise, in the middle of the exercise in the field and immediately following completion of the exercise. Prior to the first test session volunteers had an opportunity to practice these tests.

2.10.1.1 *Four-choice reaction time*

Tests of visual reaction time administered using portable laptop computers followed a procedure used previously [25, 26]. Volunteers were presented with a series of visual stimuli at one of four different spatial locations on a computer screen. They had to indicate the correct spatial location of each stimulus by pressing one of four adjacent keys on the computer keyboard. The measurements recorded included correct responses and incorrect responses (hitting the wrong key), the response latency for each study, premature errors (responding before presentation of the stimulus) and time-out errors (response latency greater than one second).

2.10.1.2 *Matching to sample test*

This test assesses short-term spatial memory (working memory) and pattern recognition skills. The volunteer responded by pressing the down arrow key when the word "ready" appeared on the screen. The volunteer was then presented for 4 seconds with an 8 X 8 matrix of a red and green checkerboard on a colour screen. The sample was removed and followed by a variable delay interval during which the screen was

blank (except for the word "delay" at the bottom of the screen). The delay was either of one or 15 seconds. After the delay, two matrices were presented on screen; the original matrix and another matrix that differed slightly, in that the colour sequence of two of the squares was reversed. The volunteer selected the comparison matrix by responding on the left or right arrow key that matched the original matrix. A comparison response (left or right arrow key) had to be made within 15 seconds, otherwise a time-out error was recorded. Correct response time to choose the matrix was also recorded.

2.10.1.3 *Visual vigilance test*

This test was developed to assess vigilance, the ability to sustain attention during a relatively boring, continuous task with minimal cognitive load. Maintenance of vigilance is essential for a variety of occupations and critical military tasks such as sentry duty, vehicle operation and monitoring surveillance and communications equipment. This test of vigilance has been shown to be sensitive to a variety of environmental and nutritional conditions, as well as sleep loss [27]. The subject is required to continuously scan the computer screen to detect the occurrence of an infrequent, difficult to detect stimulus which appeared randomly on the screen for two seconds. On average a stimulus was presented once per minute. Upon detection of the stimulus, the volunteer pressed the space bar on the keyboard as rapidly as possible. The computer recorded whether or not a stimulus was detected and the time required for correct detections. Responses made before or after stimulus occurrence were recorded as false alarms.

2.10.2 Mood and environmental symptoms

The Profile of Mood States (POMS, McNair, Lorr and Droppleman, Edits., CA USA) was used to assess mood and the Environmental Symptoms Questionnaire (ESQ, Health and Performance Division, US Army Research Institute of Environmental Medicine, Natick, USA) were used to assess the subjects' changing mood state and physical responses to the environment (Appendix D)[28]. Questionnaires were administered at baseline (day before the exercise), first day of the exercise and days 3, 6, 9 and 12 (final day) of the training exercise. The ESQ consists of 68 questions such as "I felt light headed", "I feel alert" and "I lost my appetite". There are six possible responses from "not at all" to "extreme", which were scored 0 to 5 (ie maximum possible score of 408). For the purposes of data reduction each subject was initially scored for the total 68 questions on a present or absence basis (ie total score of 68). Then symptoms were broken down into an index of subjective heat illness (light headed, headache, dizzy, faint, coordination off, short of breath, hard to breathe, heart beating fast, muscle cramp, stomach cramps, weak, constipation, warm, sweaty, body parts numb, vision blurry, lost appetite, sick, thirsty, tired, irritable and restless) and into symptoms, which have previously been associated with exercise in the heat [29] (stomach cramps, chilly, dizzy, warm, sweaty, heart beating fast, irritability, restlessness, disturbed coordination, weakness, shivering and nausea).

2.11 Statistical analyses

Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences, version 9.0, 1999, SPSS, Inc., Chicago, IL). Descriptive statistics were obtained to establish a measure of central tendency and are presented as means, standard deviations and range. Data were checked for outliers and non-homogeneity of the population by use of pair wise scatter plots, box plots and Q-Q plots.

Significance was accepted at $p < 0.05$. Multiple linear regression analyses were used to assess associations between variables. Comparison of means was achieved by use of the paired t test and Levene's test was used for comparison of variance. In order to determine the statistical difference between treatment groups, Univariate analysis (general linear model) with LSD post-test was applied to the change in response in the case of variables recorded at baseline and completion of the study. Repeated measures analysis of variance, which was based on covariance-adjusted post-treatment responses, was used to compare dietary treatments for tests with serial measurements.

3. RESULTS

3.1 Presentations for medical problems

Table 1 lists the number of presentations by each dietary treatment group for eight different medical conditions.

Table 1 Presentations for medical treatment by category & dietary treatment group

Category	Full CRP	Half CRP	Fresh	Totals
URTI	2	2	7	11
Skin problems	2	5	6	13
Joints/muscle	7	3	0	10
Injury	0	0	1	1
Dehydration	3	2	1	6
Insect bites	0	0	2	2
Exhaustion	4	1	2	7
Other	0	5	5	10
Totals	18	18	24	60

3.2 Dietary Intake

Table 2 summarises the daily nutrient intake by the three nutritional treatment groups and Table 3 presents the results of univariate analysis for each of the macronutrients and energy intakes.

Table 2 Daily macronutrient intake by nutrition treatment groups

Treatment Group	Energy (MJ)			Protein (g)			Fat (g)			Carbohydrate (g)		
	1 ^a	2	3	1	2	3	1	2	3	1	2	3
N	10	10	13	10	10	13	10	10	13	10	10	13
Minimum	6.75	5.0	10	62	48	92	68	41	97	193	160	261
25 th percentile	7.7	6.2	11	77	61	108	81	49	112	208	179	296
Median	8.2	6.8	12	90	63	115	88	53	118	231	209	331
75 th percentile	11	6.9	12	101	64	122	111	55	125	321	216	354
Maximum	12.8	7.5	14	107	74	143	122	57	150	412	227	398
Mean	9.24	6.6	12	88	63	116	94	51	121	273	199	327
S.D.	2.27	0.8	1.3	16	7.3	14	19	5.9	16	88	25	40
Std error	.72	0.2	.35	5.1	2.3	4.0	6.1	1.9	4.3	28	7.8	11
Lower 95% CI	7.61	5.9	11	76	58	108	80	47	111	210	181	303
Upper 95% CI	10.8	7.1	13	99	68	125	108	55	130	336	217	351

a 1 = Full CRP, 2 = Half CRP and 3 = Fresh group

Table 3 Comparison of macronutrient intakes between nutritional treatment groups^a

	Mean			Analysis of Variance	
	Full CRP	Half CRP	Fresh	F	P
Energy (MJ)	9.24 ± 2.27	6.55 ± 0.79	11.95 ± 1.26	35.108	<0.01
Protein (g)	87.9 ± 16.1	62.9 ± 7.3	116.4 ± 13.9	46.669	<0.01
Fat (g)	94 ± 19.3	50.7 ± 5.9	120.7 ± 15.7	62.926	<0.01
Carbohydrate (g)	273.4 ± 88.0	198.9 ± 24.7	327 ± 39.8	14.770	<0.01

^a Protected LSD post-hoc analysis indicated that there were differences for all macronutrients with treatment group.

The acceptability of the high carbohydrate containing CRP items was described by collating the percentage of these items consumed. Table 4 presents this information along with the contribution of the various high carbohydrate items to overall carbohydrate consumption by subjects in the two CRP treatment groups. Figure 2 displays the average consumption of macronutrients compared with the average amount of macronutrients available. Figure 3 illustrates the weight of the average ration consumed by each dietary treatment group.

Table 4 Acceptability of high carbohydrate-containing CRP items. Average consumption of high carbohydrate CRP items.

Group	Contribution of item to total carbohydrate available in ration (%)		Proportion of the CRP item consumed by subjects (%)		Proportion of total carbohydrate consumed (%)	
	Full CRP	Half CRP	Full CRP	Half CRP	Full CRP	Half CRP
Condensed milk	9	9	20	20	1.8	1.8
Fruits	10	10	60	91	6	9.1
Sugar (total)	20	19	44	64	8.8	12.2
white sugar			6	20		
candy			4	7		
Ergo™ drink			29	39		
Chocolate drink			5	18		
Biscuits	9	17	65	90	5.9	15.3
Muesli bars	8	8	28	86	2.2	6.9
Energy bars	21	Not offered in menu	81	Not offered in menu	29.2	
Ration						
Chocolate			7			
M & Ms			20			
HooAh!™ bar			54			
Main meals	11	15	86	94	9.5	14.1
Vegetables & starches	7	14	36	90	2.5	12.6
FD rice			1	7		
Potato & onion powder			0	20		
Noodles			16	43		
Baked beans			19	20		
Other items	5	8	44	56	2.2	4.5
Soup			2	not offered		
Beverage			20	26		
powder			22	30		
condiments						

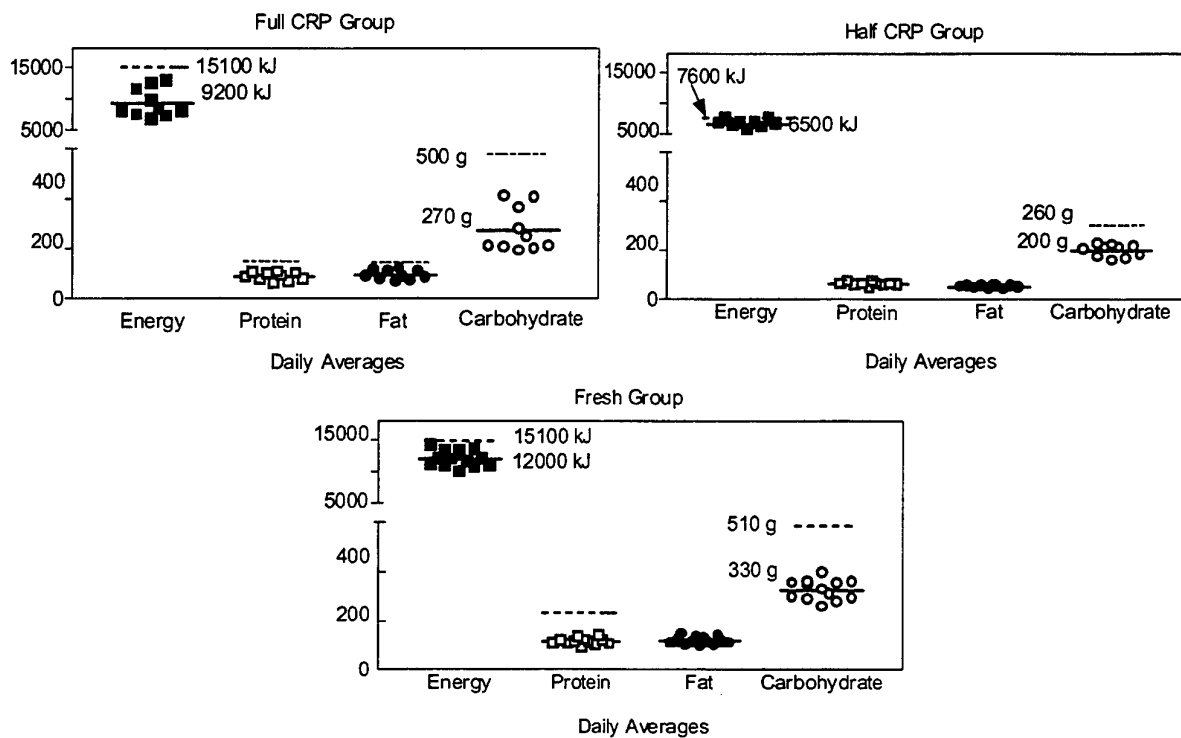


Figure 2 Average daily consumption of macronutrients by each of the three dietary treatment groups. In each graph the upper dotted line represents the average amount of the macronutrient available in the ration and the solid line represents the average macronutrient intake by the dietary treatment group.

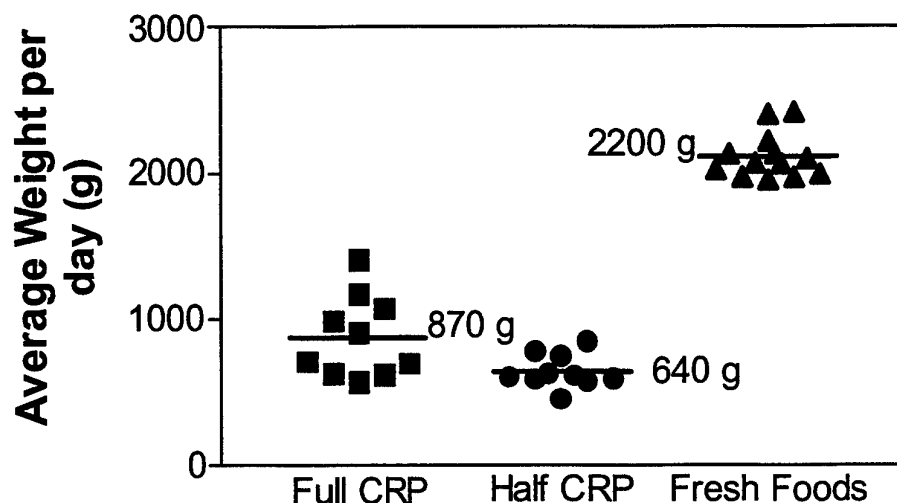


Figure 3 Average daily weight of rations consumed by each of the dietary treatment groups. The solid line is the average value.

3.3 Physiological Measurements

3.3.1 Fitness Tests

There was little change in the number of chin-ups, sit-ups or hand-grip strength recorded between the initial and final testing. Most subjects recorded an increased time for their 2.4 km run and increased hand-grip strength. The differences between the three dietary groups was not significant and the increased run times were not significant (paired t test, $t = 1.00$, 2-tailed $P = 0.324$). The increased hand-grip strength was significant for each group and for the combined groups (paired t test, $t = 7.89$, 2-tailed $P < 0.01$ for R hand grip and $t = 7.27$, 2-tailed $P < 0.01$) for the L hand grip. Table 5 details the results of the fitness testing.

Table 5 Comparison of fitness between dietary treatment groups.

	Mean change			Univariate analysis	
	Full CRP	Half CRP	Fresh	F value	p
Chin-ups	1	0	1	1.09	.35
Sit-ups	0	14	-6	2.513	.099
2.4 Km run (sec)	22	32	21	0.245	.785
Hand-grip-R (psi)	8.85	6.9	6.9	0.472	0.629
Hand-grip-L (psi)	4.3	7.8	6.6	1.484	0.245

3.3.2 Anthropometric Tests

The groups eating CRP recorded significantly lower weights, BMI, MUAC, AMA, % body fat and increased total body water, while the Fresh group recorded no significant changes in body composition. Table 6 presents the results of (significant) paired t tests for the various anthropometric measurements and Table 7 presents the results of univariate analysis of dietary treatment effect.

Table 6 Paired t tests for anthropometric measurements

	Full CRP		Half CRP		Fresh		Combined ^a	
	t	P (2-tailed)	t	P (2-tailed)	t	P (2-tailed)	t	P (2-tailed)
Weight	-2.59	0.029	-4.49	0.002	-0.33	0.749	-3.76	0.001
BMI	-2.99	0.015	-4.38	0.002	0.35	0.734	-3.20	0.003
MUAC	-4.16	0.002	-5.37	<0.01	-1.97	0.081	5.58	<0.01
AMA	-1.88	0.093	-4.46	0.002	-1.38	0.202	-3.96	<0.01
% fat - BIA	-5.03	0.001	-4.68	0.001	-0.58	0.576	-5.17	<0.01
TBW - BIA	4.72	0.002	1.11	0.296	0.004	0.997	2.50	0.019

^a Combined Groups refers to the three treatment groups, n = 31.

Table 7 Comparison of anthropometry between dietary treatment groups

	Mean change			Analysis of Variance	
	Full CRP	Half CRP	Fresh	F	P
Weight (kg)	-1.48	-1.92	-0.15	3.662	0.039
Triceps SF (mm)	-0.26	0.29	-0.20	0.314	0.733
Subscapular SF (mm)	-0.19	-0.17	0.26	0.034	0.967
Biceps SF (mm)	0.18	0.21	0.00	0.363	0.699
Suprailiac SF (mm)	-0.52	-0.21	0.33	1.138	0.335
BMI	-0.43	-0.61	0.00	4.677	0.018
MUAC (cm)	-0.42	-1.00	-0.45	3.294	0.052
AMA (%)	-3.56	-7.03	-2.13	1.585	0.223
% Fat -BIA	-20.3	-17.7	-1.7	6.738	0.005
TBW -BIA (kg)	1.1	0.4	2.2	0.499	0.613

Post hoc statistical analysis (LSD) revealed some significant differences between the dietary treatment groups. Weight and MUAC loss by the Half CRP group was significantly more than the Full CRP and Fresh groups but the difference between the Full CRP and Fresh groups was not significant. Each of the CRP groups had a significantly greater BMI and fat loss than the Fresh group, but were not distinguishable from each other.

Figures 4 and 5 present a comparison of the data collected by use of the three body composition methods, namely deuterium dilution, BIA and skinfold measurement. Table 8 presents the results of univariate analysis of data collected by the three methods.

Table 8 Comparison of body fat between the dietary treatment groups as determined by three methods.

	Mean change			Analysis of Variance	
	Full CRP	Half CRP	Fresh	F	P
% Fat (calculated from skinfolds)	2.19±7.9	0.75±7.0	-0.43±6.8	0.343	0.713
% Fat (D ₂ O dilution)	-38.7±35.1	-51.6±43.3	3.61±13.4	6.126	0.008
% Fat -BIA	-20.3±12.4	-17.7±11.8	-1.7±10.5	6.738	0.005
TBW (D ₂ O dilution) (kg)	7.54±9.9	6.83±9.2	5.75±1.3	2.069	0.149
TBW -BIA (kg)	1.1±2.0	0.4±1.2	0.0±1.6	1.031	0.371

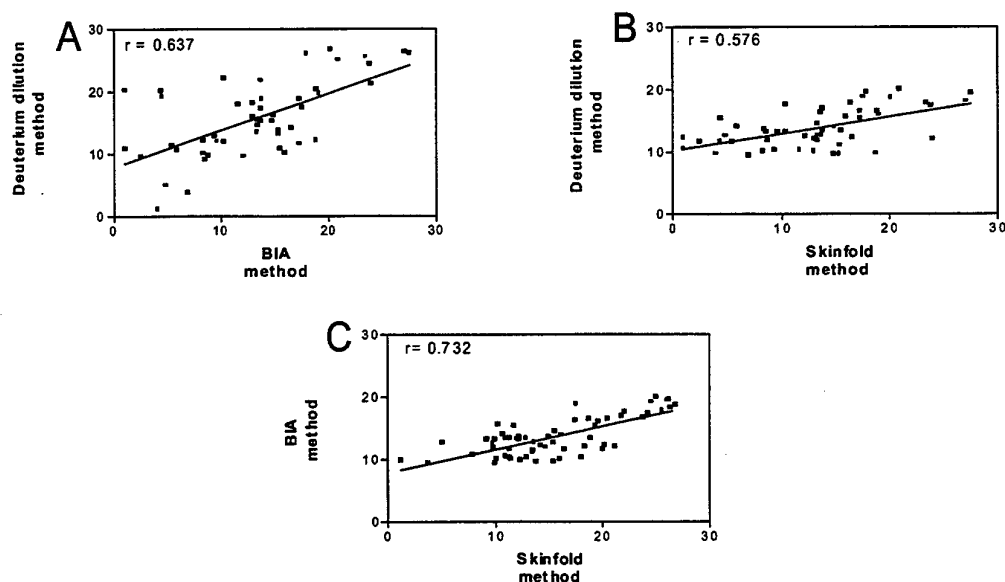


Figure 4 Comparison of %body fat results (two testing days) by three methods. The methods are described in detail in the main test. For each method comparison the correlation is significant ($p < 0.001$).

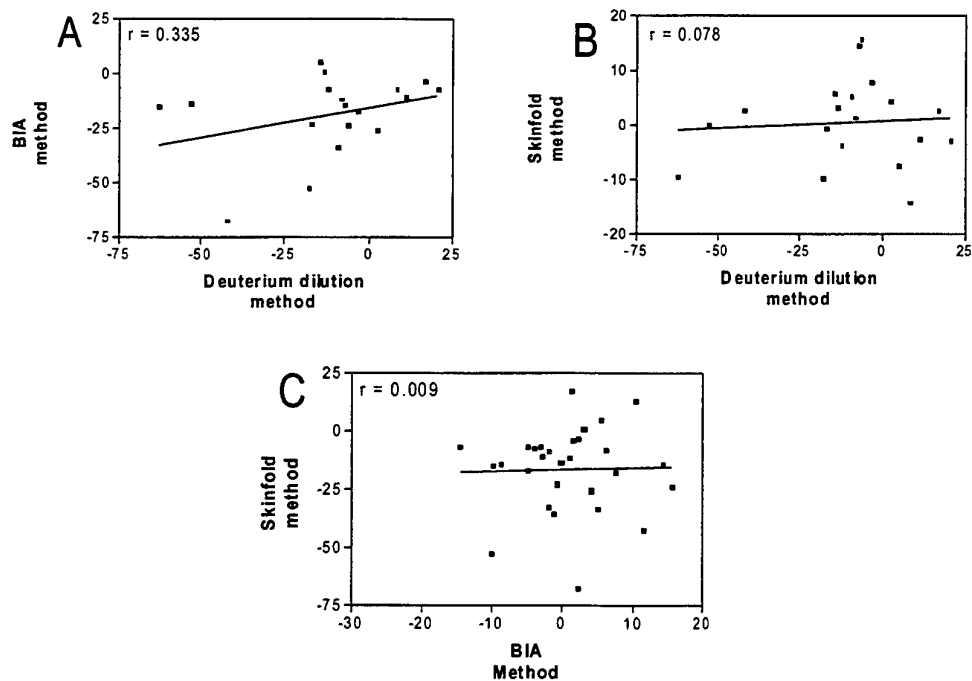


Figure 5 Comparison of %change in body fat results (two testing days) by three methods. The methods are described in detail in the main text. For each method comparison the correlation coefficient is not significant (A $p = 0.17$, B $p = 0.76$, C $p = 0.89$).

3.3.3 Total Energy Expenditure (TEE)

Average energy expenditure was determined to be 15500 ± 4400 kJ ($n = 8$). Average energy expenditures of 14600 (median 12800) kJ and 16000 (median 16200) kJ were determined for the Full CRP and Half CRP groups, respectively. Average energy expenditures ($t = 0.4179$, $p = 0.6904$) and SDs ($F = 1.113$, $p = 0.466$) were found not to differ between the two CRP groups.

3.3.4 Effect of Diet on Physical Performance

Linear regression analysis did not reveal significant relationships between dietary intake (macronutrients and energy) and physical fitness. However, Spearman rank correlation indicated a significant negative correlation between average daily carbohydrate intake and changed run times (correlation coefficient = -0.455 , $p = 0.020$) and between average daily energy intake and changed run times (correlation coefficient = -0.431 , $p = 0.028$).

3.4 DTH tests

The small difference between the positive scores (mean change) recorded by the Fresh and the combined CRP groups approached significance ($F = 3.538$, $p = 0.074$), with the Fresh group recording a smaller decrease in positive scores.

3.5 Activity measurements

The data derived from the 24-hour periods included activity counts per minute, total hours of sleep period, number of hours of sleep per night, minutes spent awake within the sleep event and the number and average duration of the sleep disturbances or awakenings after sleep onset. From these statistics additional data were derived; non-sleeping hours, number of sleep opportunities per night, sleep and wake percentages, minutes of sleep-to-wake ratio and latency to sleep onset. The quantity of sleep measures is presented in Table 9. Overall the subjects experienced a highly disruptive and poor quality of sleep during the field exercise.

Over the ten day monitoring period, the subjects averaged activity levels that were comparable with U.S. Marines performing a construction mission on Grand Inagua Island Bahamas in a hot, humid environment and Marines performing artillery training exercises at the Chocolate Mountain Warfare Training Centre, a hot dry environment [29, 30].

Across all three dietary treatment groups, the average daily activity level, measured in counts per minute (cpm) averaged 162 ± 26 . There was little difference between the treatment groups. The Full CRP group ($n = 8$) averaged 172 ± 32 over the ten days, the Half CRP group ($n = 8$) averaged 157 ± 22 cpm and the Fresh group ($n = 3$) averaged 153 ± 16 cpm. Given that the Fresh group was represented by only three subjects and there is a large variability in the data it is meaningless to attempt a comparison of physical activity.

Table 9 Quantity of sleep measures

Sleep Criteria	Full-CRP ($n = 8$)	Half CRP ($n = 8$)	Fresh ($n = 3$)
Number of days	10	10	10
Average number of sleep hours per day	4.7 ± 2.0	5.2 ± 1.3	6.0 ± 1.2
Average number of sleep opportunities per day	2.7 ± 0.8	2.7 ± 0.5	2.9 ± 0.4
Average hours per sleep opportunity	2.2 ± 1.7	2.3 ± 2.2	2.5 ± 2.2
Number of awakenings	13.8 ± 5.2	11.4 ± 3.5	12 ± 0.4
Sleep onset latency (mins)	26.3 ± 7.8	21.6 ± 4.0	23.4 ± 4.3

The differences over time and between the treatment groups were not significant. The subjects across all three dietary treatment groups averaged 5.1 ± 1.6 hours of sleep per night, distributed over an average of 2.7 ± 1.6 opportunities per night, each lasting an average of 2.3 ± 2.0 hours. The fragmentation of this sleep (measured by number of

awakenings per sleep opportunity) averaged 12.5 ± 4.1 per night for all subjects. Sleep onset latency (the amount of time recorded before a subject registers sleep) averaged 23.9 ± 6.1 minutes per night for all subjects over the ten day study period.

3.6 Biochemical Measurements

3.6.1 Urine analysis

3.6.1.1 Hydration

A high prevalence of dehydration as evidenced by urine SG measurement was recorded. However, there was no significant effect due to dietary treatment or trends over time. Figure 6 displays the proportion of subjects on a daily basis who had elevated urine SG measurements recorded (ie $SG \geq 1.030$).

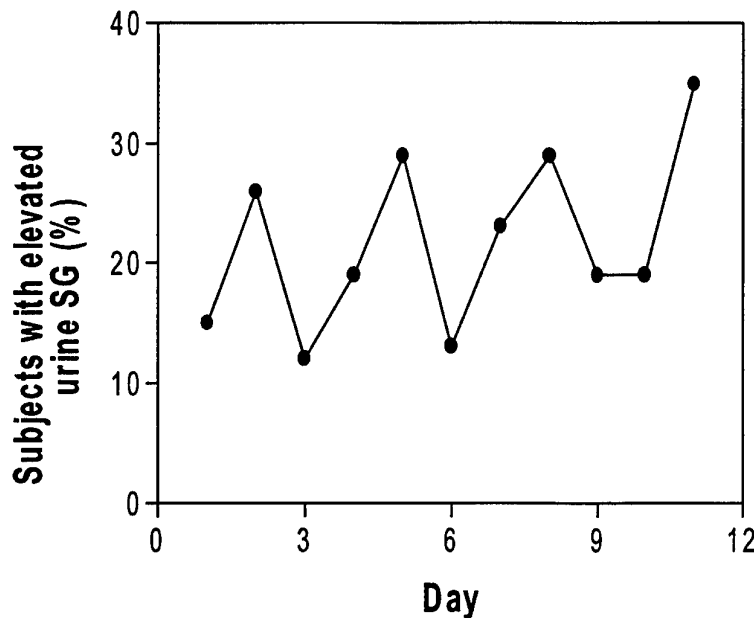


Figure 6 Prevalence of elevated urine specific gravity. Urine specific gravity values ≥ 1.03 were considered to be elevated and to indicate dehydration.

3.6.1.2 Cigarette smoking

There were 8 smokers in the Full CRP group, 6 smokers in the Half CRP group and 8 smokers in the Fresh group. Although the concentration of total nicotine metabolites (TNM) measured in urine samples of the Fresh subjects appeared less than in the other groups, the differences were not significant. Changes in TNM over the course of the exercise were not significant. Average TNM values over the study period are detailed in Table 10. Four subjects who had undetectable concentrations of TNM in their urine at baseline apparently began smoking during the field exercise. Several subjects

recorded low concentrations of TNM in their urine on occasional days and this might be due to passive smoking.

3.6.1.3 Muscle breakdown

There was no difference in the ratio of urinary 3-methylhistidine to 1-methyl histidine (3MH:1MH) between the treatment groups at baseline. Over the course of the exercise the 3MH:1MH increased ($F = 17.136$, $p < 0.01$) and there was a significant difference between the dietary treatment groups ($F=3.348$, $p = 0.038$). Figure 7 presents the covariance-adjusted means for the treatment groups at each time point. Both the Full CRP and Fresh groups recorded greater 3MH:1MH than the Half CRP group. Full CRP > Fresh ($p = 0.026$) > Half CRP ($p = 0.015$) and the Full CRP > Half CRP ($p < 0.01$). All individual results recorded were well within the expected range for healthy non-exercising adults as determined in a pilot study conducted during the previous year at DNRC. This pilot study recorded values of 1.87 ± 1.11 for subjects eating beef and lamb CRP main meals and 1.18 ± 0.71 for subjects eating their usual diet (unpublished data). The average ratio of 3MH:1MH over the 12-day study period for each dietary treatment group is detailed in Table 10.

Table 10 Average values for metabolites measured in urine

	Average results over 11 days			Analysis of Variance	
	Full CRP	Half CRP	Fresh	F	P
TNM (mg/mmol creatinine) x 10 ⁻²	28 ± 42	20 ± 21	7 ± 10	1.740	0.193
3MH:1MH	1.74 ± 0.89	1.22 ± 0.80	1.45 ± 0.86	3.805	0.037

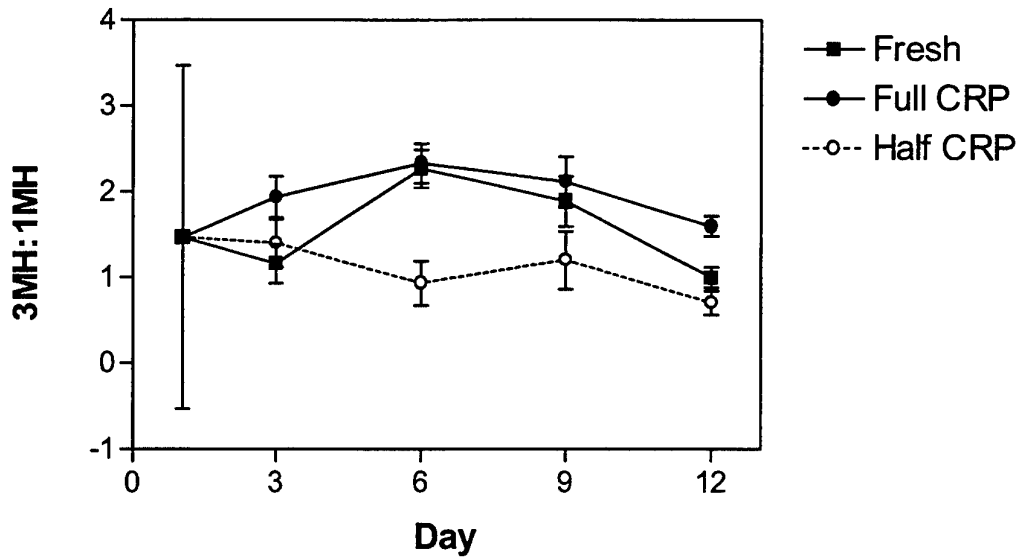


Figure 7 The urinary 3MH:1MH for each treatment group is presented as the baseline covariance adjusted mean and SD for each of the testing days. The baseline value (day 1) is presented as the average value and SD for all subjects.

3.6.2 Blood analysis

There were significant average decreases in IGF-1, fibronectin, IL6 and IL2 concentrations and increase in total antioxidant status for the CRP groups. The Fresh group experienced a significant increase in IL2r and an increase in IL6, which approached significance. Tables 12 and 13 present the results of paired t tests for these analytes and Table 11 presents the average results for all the biochemical measures.

Table 11 Average and SD for biochemistry measures

	Full CRP		Half CRP		Fresh	
	First day	Last day	First day	Last day	First day	Last day
IGF-1 (ng/L)	262 ± 104	176 ± 79	225 ± 48	135 ± 42	235 ± 87	207 ± 67
CMI-positives	3.6 ± 2.1	3.7 ± 2.3	3.4 ± 1.5	2.8 ± 1.5	3.4 ± 1.7	3.8 ± 1.9
Homocysteine (umol/L)	11.2 ± 2.5	12.8 ± 4.0	12.2 ± 5.4	13.0 ± 4.2	10.4 ± 4.4	10.9 ± 2.9
Total Antioxidant Status (mmol/L)	1.34 ± 0.11	1.7 ± 0.34	1.35 ± 0.08	1.58 ± 0.30	1.42 ± 0.10	1.54 ± 0.34
Ferritin (g/L)	133 ± 72	121 ± 80	151 ± 81	137 ± 78	151 ± 76	104 ± 54
Fibronectin (g/L)	286 ± 138	250 ± 42	301 ± 134	219 ± 23	296 ± 159	281 ± 82
CRP (g/L)	5.0 ± 5.9	1.2 ± 1.2	2.8 ± 1.6	301 ± 3.1	3.0 ± 1.4	4.4 ± 6.6
PIVKA (ng/ml)	3.78 ± 1.74	4.52 ± 1.95	4.60 ± 1.36	4.32 ± 1.53	3.55 ± 1.54	4.45 ± 1.32
IL2R (U/mL)	306 ± 82	350 ± 130	336 ± 79	376 ± 161	307 ± 95	451 ± 173
IL6 (ng/mL)	1.8 ± 0.8	7.7 ± 4.8	1.2 ± 0.5	9.9 ± 5.6	1.2 ± 0.5	7.2 ± 3.4
IL2 (ng/mL)	13.4 ± 8.7	9.9 ± 8.7	13.6 ± 5.5	9.2 ± 3.7	10.4 ± 6.2	9.4 ± 3.7

Table 12 Results of paired *t* tests for each of the dietary treatment groups

	Full CRP (n = 10)		Half CRP (n = 10)		Fresh (n = 11)	
	t	P (2-tailed)	t	P (2-tailed)	t	P (2-tailed)
IGF-1	-3.1	0.013	-8.9	<0.01	-1.23	0.246
Total Antioxidant Status	3.04	0.014	2.75	0.022	0.958	0.361
Ferritin	-0.9	0.373	-1.0	0.358	-1.03	0.329
IL2r	1.08	.307	1.01	.337	2.653	.024
IL6	-2.8	0.026	-4.0	0.004	1.887	0.088
IL2	-2.9	.018	-3.4	.007	-0.50	.627

Table 113 Results of paired *t* tests for combined dietary treatment groups

	Combined groups (n= 31)		Combined CRP groups (n = 20)	
	t	P (2 tailed)	t	P (2 tailed)
IGF-1	-5.106	<0.01	-6.149	<0.01
Total	3.614	.001	4.077	0.001
Antioxidant Status				
Ferritin	-1.742	.092	-1.382	0.183
Fibronectin	-1.898	.067	-2.089	0.05
IL2r	2.876	.007	1.523	0.144
IL6	-1.276	.0213	-0.811	0.427
IL2	-3.142	.004	-4.572	<0.01

Subjects eating CRP (ie combined CRP group) experienced significant decreases in IGF-1 ($F=15.771$, $p < 0.01$), fibronectin ($F = 4.861$, $p = 0.038$), IL2 ($F = 4.556$, $p = 0.044$) and IL6 ($F = 16.96$, $p < 0.01$) concentrations when compared with the Fresh group. In each case the changes were not significantly different between the two CRP groups. Table 14 presents the results of univariate analysis of dietary treatment effect. Figure 8 displays the change in fibronectin and IGF-1 concentrations as scatter plots. In the case of both fibronectin and IGF-1 the Fresh group experienced no significant change in status.

Table 14 Comparison of blood chemistry between dietary treatment groups

	Mean changes (%)			Analysis of Variance	
	Full CRP (n = 10)	Half CRP (n = 9)	Fresh (n = 11)	F	P
IGF-1	-28 ± 24	-35 ± 5	-4 ± 17	8.084	0.002
Total	29 ± 30	18 ± 20	10 ± 29	1.364	.273
Antioxidant Status					
Ferritin	-9 ± 32	-1 ± 38	-8 ± 30	0.370	0.694
Fibronectin	-10 ± 58	-40 ± 12	18 ± 61	2.935	0.071
^a CRP	-54 ± 36	-1 ± 92	-7 ± 49	2.065	0.146
PIVKA	25 ± 39	-1 ± 43	19 ± 37	1.120	0.341
SIgA:Alb	-35 ± 56	-48 ± 34	-15 ± 53	1.162	0.327
day 6					
IL2r	19 ± 42	11 ± 37	57 ± 75	2.101	0.141
IL6	-32.6 ± 23.6	-12.9 ± 7.6	15.7 ± 29.5	10.730	<0.01
IL2	-34 ± 15	-39 ± 10	-18 ± 30	2.288	0.125

^a CRP, C-reactive protein measurements were all below 15 g/L and therefore subjects were assessed to be free of active infective illness or chronic inflammation [31].

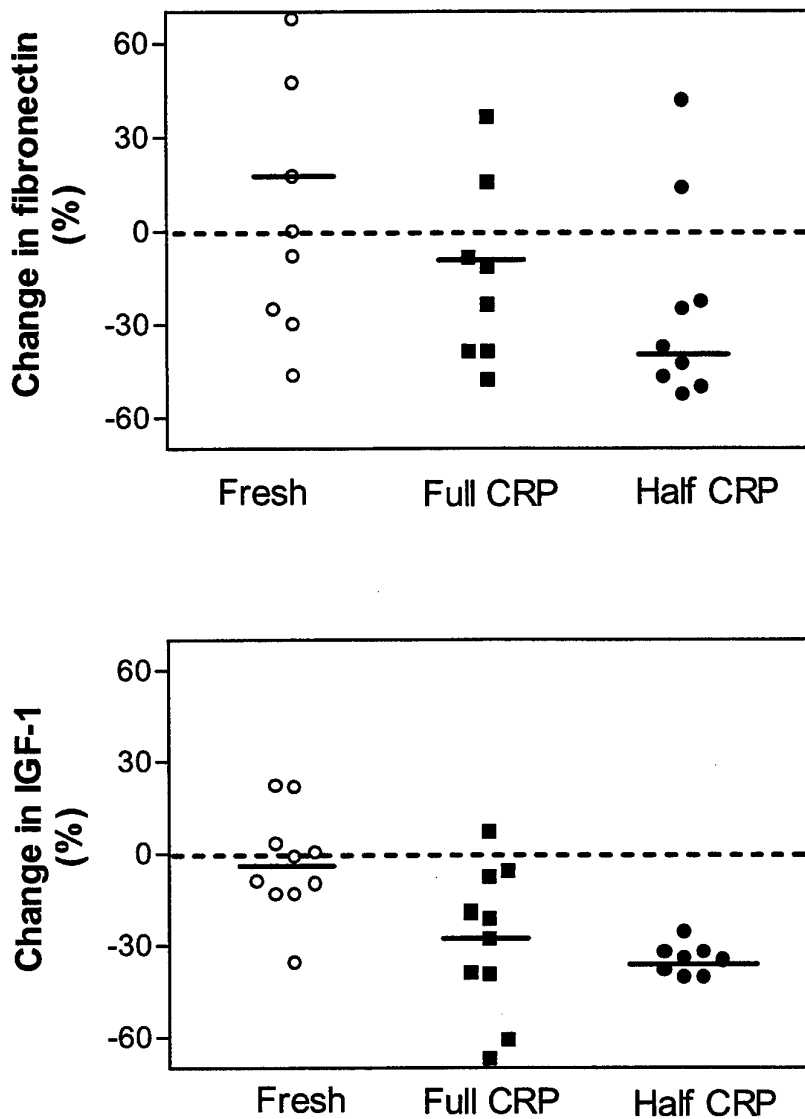


Figure 8 Plasma fibronectin and serum IGF-1 are expressed as the percentage change in concentration over the 12 day study period. The Fresh group maintained IGF-1 ($t = -1.23$, $p = 0.246$) and fibronectin ($t = -0.38$, $p = 0.713$) status, while the combined CRP group ($n = 19$) had a significant decrease in IGF-1 ($t = -6.149$, $p < 0.01$) and fibronectin ($t = -2.089$, $p = 0.05$) status. The combined CRP group had a significant change in IGF-1 ($F = 15.771$, $p < 0.01$) and fibronectin ($F = 4.861$, $p = 0.038$) compared with the Fresh group, but in each case there was no significant difference between the two CRP groups. The mean for each scatterplot is indicated as a solid line.

Vitamin status was determined by measurement of total plasma antioxidant capacity, total plasma homocysteine (Hcys) and concentration of circulating PIVKA II. Table 15 describes the percentage of subjects who had values outside the healthy clinical range.

On the initial day of testing 12 subjects had elevated Hcys concentrations (ie > 10 $\mu\text{mol/L}$, an indicator of folate deficiency) and after the study 22 subjects had elevated Hcy concentrations. Because the Hcys results were skewed to elevated values, a non-parametric test was used to compare differences. Although not significant the median Hcy concentrations increased in the order Half-CRP group > Full-CRP group > Fresh group. The increase in Hcys concentration for the combined groups ($n = 31$) was significant (Wilcoxin Signed Ranks test, $Z = 2.236$, $p = 0.025$).

All subjects had sub-optimal antioxidant status on the initial testing day. During the course of the exercise antioxidant status improved for the Full CRP ($t = 3.04$, $p = 0.014$) and the Half CRP ($t = 2.75$, $p = 0.022$) groups and remained stable for the Fresh group ($t = 0.958$, $p = 0.361$).

Initial PIVKA II concentrations for all subjects were $3.96 \pm 1.57 \mu\text{g/L}$ (range 1.41 – 7.5) and final testing-day concentrations were 4.43 ± 1.56 (range 1.82 – 7.91). The average increase in PIVKA II for the combined group was not significant ($t = 1.7$, $p = 0.099$). The trend was for PIVKA to increase in the Full CRP group ($t = 1.9$, $p = 0.09$) and Fresh group ($t = 2.03$, $p = 0.07$) and to remain unchanged in the half CRP group ($t = -0.53$, $p = 0.61$). The differences between the dietary treatment groups were not significant ($F = 1.12$, $p = 0.341$). It was noteworthy that the vitamin K status of these ADG's was poor. Nearly all the subjects had higher than normal PIVKA II concentrations.

Table 15 Proportion of subjects outside the optimal range for functional measures of vitamin status.

	Clinical Cut-off	Proportion outside clinical range (%)					
		Full CRP		Half CRP		Fresh	
		Day 1	Day 12	Day 1	Day 12	Day 1	Day 12
Total antioxidant capacity (mmol/L)	<1.2[32]	100	40	100	50	92	45
Total homocysteine ($\mu\text{mol/L}$)	>10.0[33]	50	70	40	70	45	73
PIVKA II ($\mu\text{g/L}$)	> 2.0[34]	90	90	100	90	82	100

3.6.3 Saliva analysis

The differences in average saliva IgA:albumin between the treatment groups at baseline were not significant. The decrease in the saliva IgA:albumin over the course of the training exercise was significant ($F = 5.219$, $p = 0.007$). The greatest decrease from baseline occurred on day 3 ($p = <0.01$) and the Fresh group had a boost in saliva IgA:albumin on day 9 ($p = 0.017$). Table 16 details the average results for each testing

day for the three dietary treatment groups and Figure 9 shows the percentage change in results for each of the dietary treatment groups over the course of the exercise.

Table 16 Average saliva IgA:albumin results for five testing days

	Mean		
	Saliva IgA/albumin		
	Full CRP (n = 10)	Half CRP (n = 10)	Fresh (n = 9)
DAY 0	3.61 ± 2.68	3.98 ± 1.54	4.86 ± 3.3
DAY 3	2.27 ± 2.00	1.96 ± 1.31	2.93 ± 1.55
DAY 6	2.21 ± 2.37	1.96 ± 1.21	3.13 ± 2.1
DAY 9	1.87 ± 1.71	2.63 ± 1.71	4.92 ± 1.76
DAY 12	3.08 ± 2.73	2.24 ± 1.37	4.29 ± 2.34
Average over 12 days	2.61 ± 1.96	2.55 ± 0.96	3.81 ± 1.71

There were significantly different effects due to the three dietary treatments ($F = 2.636$, $p = 0.027$). The Fresh group had significantly higher average saliva IgA: albumin values than the combined CRP groups ($F = 3.168$, $p = 0.043$) and the half CRP group, alone ($p = 0.04$). The two CRP groups did not have significantly different sIgA: albumin.

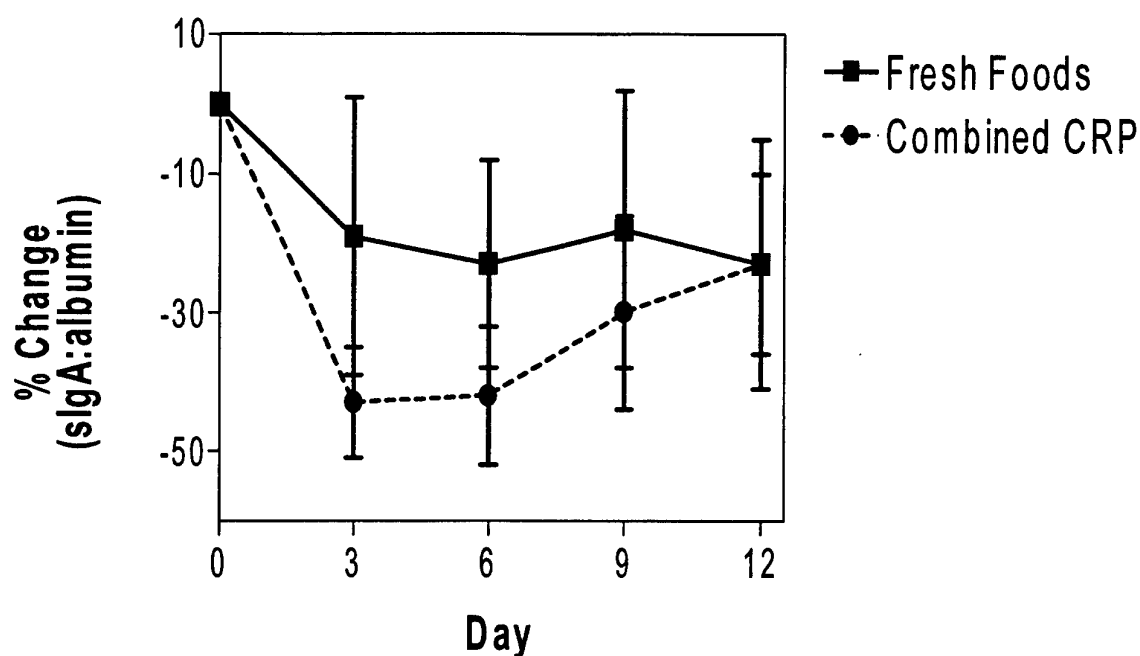


Figure 9 Percentage change in salivary IgA to albumin ratio for the Fresh and combined CRP groups over 12 days. The differences in average saliva IgA:albumin at baseline were not significant. The effect of the three dietary treatments was significant ($F = 2.636$, $p = 0.027$). The Fresh group had significantly higher average saliva IgA: albumin values than the combined CRP groups ($F = 3.168$, $p = 0.043$) and the Half CRP group, alone ($p = 0.04$). The two CRP groups did not have significantly different sIgA: albumin.

3.7 Biochemical and physiological predictors of macronutrient intake

The single best indicator of reduced macronutrient intake was the percentage change in serum IGF-1. The adjusted R squared values for average daily intakes of energy, protein, carbohydrate and fat were 0.61, 0.60, 0.47 and 0.58, respectively. A linear model incorporating the percentage change in serum IGF-1 and average sIgA:albumin (over 12 days) was a slightly better predictor of reduced energy and protein intake, with adjusted R squared values of 0.66 and 0.69, respectively. The prediction equations for energy intake were:

$$\text{Energy (kJ)} = 11417(520) + 105(17) \times \% \text{ change in serum IGF1; and}$$

$$\text{Energy (kJ)} = 9958(848) + 97(16) \times \% \text{ change in serum IGF1} + 440(210) \times \text{Average sIgA:Alb}$$

where the values in parentheses are the standard errors.

Table 17 presents the Pearson correlation coefficients for various biochemical and physiological measurement compared with macronutrient intake.

Table 17 Pearson correlation coefficients for dietary intake and biochemical and physiological measurements

	Average daily dietary intake			
	Energy	Protein	Carbohydrate	Fat
IGF 1 ^c	0.793 ^a	0.785 ^a	0.720 ^a	0.770 ^a
BMI	0.630 ^a	0.540 ^a	0.628 ^a	0.590 ^a
Weight	0.630 ^a	0.531 ^a	0.624 ^a	0.597 ^b
MUAC	0.525 ^a	0.484 ^a	0.532 ^a	0.544 ^a
IL2r	0.452 ^a	0.395 ^b	0.419 ^b	0.427 ^b
Saliva	0.423 ^b	0.480 ^a	0.394 ^b	0.359 ^b
IgA:Alb ^d				
Fibronectin	0.373 ^b	0.402 ^b	0.406 ^b	0.352 ^b
IL2	0.363 ^b		0.358 ^b	
PIVKA	0.336 ^b	0.380 ^b		0.352 ^b
IL6	0.331 ^b	0.329 ^b		
Urine				
Specific gravity ^c			0.364 ^b	

^a $p < 0.01$ ^b $p < 0.05$ ^c Presented as the percentage change. ^d Presented as the average result over 12 days.

3.8 Effect of cigarette smoking

TNM results were ranked then assigned a score of 1 to 3, where non-smokers (TNM = 0) = 1, TNM < Median = 2 and TNM ≥ median = 3. Multinomial logistic regression analysis revealed some weak evidence that cigarette smoking might be associated with immune suppression and greater weight loss. Table 18 presents the results of the statistical analysis.

Table 18 Multinomial logistic regression analysis of urinary TNM concentration against biochemical and physiological measures.

Variable	Chi Square score	Significance
Average salivary IgA	3.264	0.0708
IL2 (on last testing day)	2.9998	0.0833
Weight loss	4.842	0.089
BMI loss	4.856	0.088

3.9 Psychological measurements

3.9.1 Cognitive testing

3.9.1.1 *Four-choice reaction time*

Reaction time tasks are susceptible to the stress imposed by lack of sleep and the external environment. All subjects ($n = 31$) performed similarly for reaction time across the three testing sessions, averaging 507 ± 80 milliseconds (ms) for correct response latency. The Half CRP group ($n = 10$) averaged non-significantly faster (485 ± 55 ms) than both the Fresh group ($n = 11$, 516 ± 76 ms) and the Full CRP group ($n = 10$, 518 ± 105 ms). No significant differences were detected for any of the recorded measures previously mentioned either over time or as a result of dietary treatment.

3.9.1.2 *Match- to-sample test*

This test assesses short-term memory (working memory) and pattern recognition skills. All subjects ($n = 31$) performed similarly for correct match-to-sample comparisons across the three testing sessions, averaging 12.1 ± 1.5 correct matches. The Full CRP group had the highest average (non-significant) of 12.2 ± 1.5 correct matches, with the Half CRP group averaging 12.1 ± 1.6 and the Fresh group averaging 12.0 ± 1.5 . No significant differences were detected for any of the recorded measures (choice time, correct matches, and total incorrect responses) either over time or as a result of dietary treatment.

3.9.1.3 *USARIEM visual vigilance test*

By averaging 8.8 ± 0.9 correct hits across the three testing sessions, all subjects again performed similarly for visual detection of presented stimuli. The Half CRP group recorded the most hits (9.0 ± 0.9) with the Full CRP group averaging 8.9 ± 1.1 and the Fresh group averaging 8.5 ± 0.7 . No significant differences were detected for reaction time latency. Table 19 details the average scores for the cognitive test parameters.

3.9.2 Profile of Mood States (POMS)

Figure 10 displays the average scores for each POMS measure for the three dietary treatment groups over the 12 days. There were no significant differences in the POMS factors between the treatment groups at baseline. Over the course of the exercise there was a significant increase in the factors fatigue ($F = 13.366$, $p < 0.01$) and confusion ($F = 5.497$, $p < 0.01$) and there was a significant decrease in the factor vigour ($F = 8.873$, $p < 0.01$). Both the Full CRP group ($p = 0.048$) and the Half CRP group ($p = 0.028$) recorded greater fatigue than the Fresh group and the differences between the treatment groups for the factors vigour and confusion were close to significant ($F = 2.388$, $p = 0.078$; $F = 5.497$, $p = 0.067$, respectively). Although the CRP groups recorded increased feelings of depression-dejection on days 3 ($F = 3.768$, $p = 0.062$) and day 9 ($F = 4.402$, $p = 0.045$), overall the change with time is not significant. The

Table 19 Average scores for the cognitive test parameters

	Full CRP (n = 10)	Half CRP (n = 10)	Fresh (n = 11)	F	P
<u>Four-choice reaction time</u>					
Correct Response Latency (ms)	518± 105	486±55	516±76	0.494	0.615
Premature Hits	0.4±0.7	0.4± 0.7	0.4±0.7	0.033	0.967
Time-Out Errors	1.7± 3.8	0.3±0.4	0.2±0.3	1.579	0.224
<u>Matching-to-Sample</u>					
Choice Time (sec)	4.2±0.8	3.7± 0.6	3.7± 0.9	1.768	0.189
Correct Matches	12.2± 1.5	12.1± 1.6	12.0± 1.5	0.049	0.952
Total Incorrect Responses	2.8± 1.5	2.9± 1.6	3.0±1.5	0.049	0.952
<u>Visual Vigilance</u>					
Correct Hits	8.9± 1.1	9.0± 0.9	8.5± 0.7	1.072	0.356
Reaction Time (sec)	1.0±0.2	0.9± 0.1	1.0± 0.1	2.027	0.151

increased depression-dejection recorded by the combined CRP groups when compared with the Fresh group was almost significant ($F = 2.001$, $p = 0.066$). The factors, tension-anxiety ($r = -0.113$, $p = 0.08$) and vigour-activity ($r = 0.27$, $p = 0.003$) were associated with sIgA:Alb.

Some mood measures recorded on the last day of testing correlated significantly with macronutrient intake. Table 20 presents the Pearson correlation coefficients for measures of mood against average macronutrient intake. Tension-anxiety, anger-hostility, fatigue and confusion were negatively associated with dietary intake and vigour was positively associated with dietary intake.

Table 20 Pearson correlation coefficients for average daily dietary intake and measures of mood state^a.

	Energy	Protein	Carbohydrate	Fat
Tension-anxiety	-0.300		-0.322	-0.345
Anger-hostility		-0.310		
Vigour	0.380		0.334	0.405
Fatigue	-0.367	-0.333	-0.398	-0.338
Confusion	-0.339		-0.373	-0.359

^a All correlations have significance $p < 0.05$.

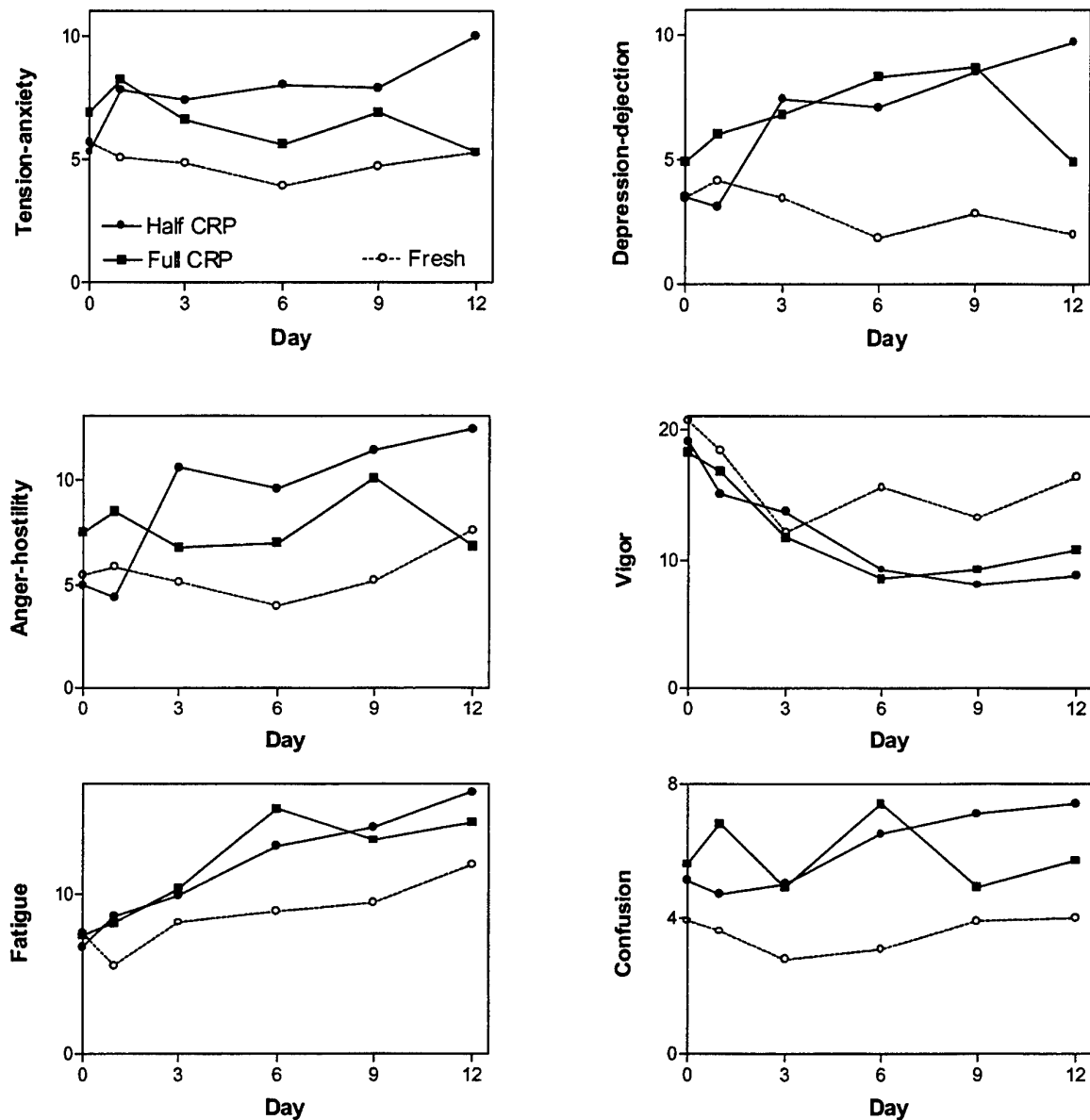


Figure 10 Average scores for POMS measures. There were no significant differences in the POMS factors between the treatment groups at baseline. Over the exercise there was a significant increase in the factors fatigue ($F = 13.366$, $p < 0.01$) and confusion ($F = 5.497$, $p < 0.01$) and there was a significant decrease in the factor vigour ($F = 8.873$, $p < 0.01$). Both the Full CRP group ($p = 0.048$) and the Half CRP group ($p = 0.028$) recorded greater fatigue than the Fresh group.

3.9.3 Environmental Symptoms Questionnaire (ESQ)

The differences in recorded symptoms at baseline were not significant. The increase in recorded symptoms over the course of the exercise was close to significant ($F = 2.313$, $p = 0.084$). The largest increase in symptoms was recorded by the Half CRP group on day 3. The differences between the treatment groups were not significant. Table 21 details the average total scores for each dietary treatment group.

Table 21 Average total scores for 68 questions by dietary treatment group and day

	Mean		
	Total scores for 68 questions ^a		
	Full CRP (n = 10)	Half CRP (n = 10)	Fresh (n = 11)
Baseline	28 ± 16	38 ± 22	33 ± 19
DAY 1	30 ± 11	42 ± 31	40 ± 27
DAY 3	41 ± 16	66 ± 34	43 ± 14
DAY 6	45 ± 14	63 ± 36	42 ± 17
DAY 9	53 ± 35	64 ± 42	46 ± 29
DAY 12	42 ± 15	63 ± 40	40 ± 18

^a Total possible score was 408

All subjects recorded increased symptoms related to exercise in the heat as the exercise progressed then on the final testing day, during which subjects were involved in little exercise, fewer symptoms were recorded ($F = 9.617$, $p < 0.01$). There was a non-significant trend for subjects in the Half CRP group to record more symptoms than the Full CRP group ($p = 0.078$) and more symptoms than the Fresh group ($p = 0.068$). These heat-related symptoms had a (non-significant) negative association with sIgA:Alb ($r = -0.107$, $p = 0.09$). Table 22 details the average scores for symptoms related to exercise in the heat.

Most subjects recorded low scores for the index of subjective heat illness. Average scores for the dietary treatment groups ranged from 0 to 8.5 (possible maximum score of score of 132).

The response to the question, "I feel hungry" was analysed over time and between subjects. Subjects became increasingly hungry as the exercise progressed ($F = 13.48$, $p < 0.01$). There was a boost in hunger on day 3. The differences in hunger between the treatment groups were significant ($F = 2.761$, $p = 0.013$). Subjects in the Half CRP group were "more hungry" than subjects in both the Full CRP group ($p = 0.023$) and the Fresh group ($p < 0.01$). There was no difference in response between the Fresh and Full CRP groups. Table 23 details the average scores for this question.

Table 22 Average total scores for questions related to exercise in the heat by dietary treatment group and day^a

	Mean		
	Symptoms associated with exercise in the heat		
	Full CRP (n = 10)	Half CRP (n = 10)	Fresh (n = 11)
Baseline	3.9±3.3	4.5±5.6	3.1±4.1
DAY 1	4.0±2.1	8.5±9.7	5.5±4.8
DAY 3	8.1±5.5	14.6±9.7	10.3±4.8
DAY 6	6.9±3.7	12.5±9.7	8.5±4.6
DAY 9	10.0±10.2	13.2±9.5	7.2±6.0
DAY 12	4.8±3.0	8.4±8.1	3.8±3.3

^a Total possible score was 360

Table 123 Average total scores for "I feel hungry" by dietary treatment group and day^a

	Mean		
	Full CRP	Half CRP	Fresh
Baseline	2.0±1.6	1.8±1.2	1.8±1.9
Day 1	0.2±0.4	0.8±0.8	0.8±1.3
Day 3	2.0±1.9	2.9±1.9	1.2±1.3
Day 6	1.9±1.6	3.3±1.6	0.7±0.9
Day 9	1.9±2.0	3.5±1.8	0.9±1.1
Day 12	2.9±2.3	2.7±2.2	1.5±2.1

^aTotal possible score was 30

4. DISCUSSION

4.1 Non-dietary effects

There was a high rate of cigarette smoking with only six subjects being non-smokers. The rate of smoking appeared to increase as the exercise progressed. For example, four subjects who had not smoked at RAAF Amberley (baseline testing day) were smoking by the third day of the exercise. There was some weak evidence that smoking suppressed immune system function and exacerbated weight loss, as evidenced by the negative association between urinary TNM concentration and salivary sIgA:albumin and weight (Table 18). The level of smoking was not significantly different between the dietary treatment groups (Table 10). The effect of diet and smoking on sIgA:albumin and weight cannot be distinguished. To our knowledge this is the first time smoking has been evaluated in an Australian field study and these possible effects need further investigation.

There was a high rate of dehydration as evidenced by the prevalence of urine SGs above 1.03 (Figure 6). Furthermore there were six presentations by subjects for medical treatment related to dehydration (Table 1). The authors believe that the high rate of dehydration indicates a need for education on the importance of maintaining a state of euhydration, because the ADGs involved in this training exercise had ready access to cached water sites.

There was a high rate of presentations for medical treatment (Table 1). The authors also noticed that many of the subjects were asking for pain relief for treatment of headaches (data not recorded). The most prevalent medical complaints appeared to be related to the environmental conditions (ie extreme humidity and heat) the most common complaint being skin rashes.

Overall the subjects experienced a highly disruptive and poor quality of sleep during the field exercise (Table 9) and reported increasing levels of environmental symptoms (Table 21), fatigue, confusion and loss of vigour (Figure 10) as the exercise progressed. No significant difference in quality or quantity of sleep or activity was found between the dietary treatment groups. The average energy expenditure of the two CRP groups was shown to be similar. Although many subjects had increased run times (20 to 30 secs) during their final physical fitness test, overall there was no significant change in the average physical fitness and no difference in fitness between dietary treatment groups.

Excretion of urinary 3MH:1-MH increased during the exercise and differences between the dietary treatment groups were significant. Because the results were

within the range determined for a group of sedentary individuals on similar ration pack diets, these differences are believed to be due to dietary composition rather than changes in muscle protein breakdown.

4.2 Dietary treatment effects

In general the Fresh subjects fared very well nutritionally. They did not lose weight and they maintained their nutritional and immune status. Furthermore there was no alteration in overall physical or cognitive performance. It can be concluded that the average nutritional requirement of 15000 kJ predicted by REAP™ was adequate to maintain nutritional status during the exercise. The determined average total energy expenditure of 15500 kJ is consistent with this view. Significant changes noted for this group included a decrease over time for salivary IgA, an increase in self-reported fatigue, loss of self-reported vigour and symptoms related to exercise in the heat.

Because subjects in the CRP groups ate less food than the Fresh group they fared less well than the Fresh group, with significant weight loss (as body fat), catabolism of body protein, mild suppression of immune function and greater reporting of fatigue, loss of vigour and more symptoms related to working in the heat. Furthermore, these subjects were more likely to note that they were "feeling hungry". There are some indications that either the restricted ration is more efficient or that subjects generally learn to adapt to CRP, because there were no significant differences in nutritional status measures between the Full and Half CRP groups despite the differences in dietary intake.

Although subjects consuming CRP had some negative biochemical and physiological indicators, there is no evidence to support the proposition that fresh foods per se confer a nutritional advantage. Importantly despite the higher intake of nutrients and the maintenance of weight in the Fresh group, there were no substantial differences among the treatment groups in their mental or physical performance. This implies that soldiers can manage on their nutritional reserves or can adapt to using nutrients more efficiently.

4.2.1 Dietary intake

The most important finding was the high rate of ration pack discards. Because the items most likely to be discarded were those high in carbohydrate, the practice resulted in many of the subjects eating insufficient carbohydrate (Tables 2 & 3) compared with that recommended for soldiers involved in routine patrolling (Appendix B and C)[1]. Even the Fresh group failed to eat all the carbohydrate available in their menu, because they discarded many of the high carbohydrate items provided in their brew kit. The brew kit included refined sugar items, biscuits and an energy bar (Appendix B). It was noted that few of the subjects made cups of tea or coffee. This was most likely the reason that 80% of the condensed milk was discarded.

Figure 2 shows that the subjects in the Full CRP group on average ate 60% of available energy in their ration menu and 55% of the available carbohydrate. Most of the carbohydrate in the Full CRP menu was provided by refined sugars (sugar, candy, Ergo™ drink, chocolate drink) and energy bars (ration chocolate, M&Ms, HooAh!™ bar). The subjects discarded more than 90% of the refined sugar items and 20% of the energy bars. Of the items eaten the major contribution to daily carbohydrate intake was from the energy bars, in particular the HooAh!™ bar.

Subjects' energy intake was proportionally high in the Half CRP vs Full CRP groups (87% vs 60%) as was the carbohydrate consumption (82% vs 56%). The implication is that subjects in the Half CRP group wasted less food when constrained to consume a more restricted ration and that they were more selective in choosing what to consume. Subjects in the Half CRP group were less likely to discard fruit-based items, biscuits, muesli bars and noodles than the subjects presented with a full CRP menu (Table 4). Energy bars were not offered in this group's menu.

The items with significant carbohydrate content, which had the greatest acceptance in terms of actual consumption, were the energy bars, fruits (fruit grains and tinned fruit), biscuits and main meals. It should be noted that this study included several items which were not routinely available in Australian CRP at the time, namely dried fruit grains and the US Army's Ergo™ drink and HooAh!™ bar. Fruit grains have since been included in CR1M and PR1M. Comments regarding the acceptability of these two products are included in Appendix E.

Subjects were not questioned about the reasons for their food choices. Historically, load carriage (both weight and ease of packing) has been cited as an important consideration in the choice of rations. Figure 3 shows the average daily weight of rations eaten by the three treatment groups. It is clear that fresh food menus weigh more than CRP.

The nutritional importance of eating sufficient carbohydrate is evidenced by the linear relationship between average carbohydrate consumption and concentration of serum IGF-1, which is a marker for protein catabolism and the negative correlation between increased run times and average carbohydrate consumption. Although not reaching significance, there was a trend for longer run times at the end of the exercise, with the half ration group recording the greatest increases.

4.2.2 Body composition

As expected, subjects fed with freshly cooked food maintained body weight and anthropometric characteristics, while the two CRP groups experienced significant losses. It had also been anticipated that, with respect to anthropometry, subjects receiving the full CRP would be midway between the Fresh and those fed with half CRP. However, this was not consistently found. Although weight and MUAC did

conform to these expectations, body fat lost by Full CRP subjects was similar to that of the Half CRP group and significantly greater than for Fresh.

This is consistent with the finding that the Full CRP group discarded 40% of their available food. The possible reasons for discarding have been discussed previously, but the anthropometric and food intake findings provide support for the recommendation by Forbes-Ewan [2] that consideration be given to the development of a lightweight, high-carbohydrate ration pack that provides for a reduced energy intake—but high carbohydrate consumption—during short term operations.

The opportunity was also taken on this study to conduct a comparison of methods of analysis of body composition. BIA, skinfolds and deuterium dilution were all used to estimate body fat. Under laboratory conditions, each method is capable of detecting moderate changes in body fat levels. However, under the less-than-ideal conditions of a field study, it appears that any change in body composition was too small to be detected by the skinfolds method. The BIA and deuterium methods picked up small changes but are also associated with large error. The magnitude of the error is illustrated in Table 8 and Figures 4 and 5. It is disappointing that the percentage change in body fat calculated by the three methods do not correlate, but this probably demonstrates the difficulty in measuring body composition in the field. Deuterium dilution, which is an expensive method, provided no better results than the cheaper and more convenient methods, so it will not be used in future field studies.

4.2.3 Vitamin status

Subjects appeared to be at risk of poor folic acid, vitamin K and antioxidant status (Table 15). Over the course of the exercise there was a significant decrease in folic acid status (as measured by total homocysteine) and an increase in total antioxidant status (as measured by total antioxidant capacity of plasma). There was a non-significant decline in vitamin K status (as measured by PIVKA II).

The laboratory methods chosen to determine folic acid and vitamin K status were functional assays, which are believed to be most representative of long-term changes (ie not within-day changes). These assays will be discussed in a review paper which is under preparation. The poor vitamin status of these subjects before the exercise suggests that their usual diet needs improvement. It is of concern that the folic acid and vitamin K status declined whilst eating CRP, particularly in light of the fact that this group had spent up to 20 weeks on exercise in the previous year and had mostly consumed CRP as their sole nutrition during that time. Both mild vitamin K and folic acid deficiency have been implicated in increased risk of coronary vascular disease [33, 35].

Reactive oxygen species are either synthesized endogenously for example in energy metabolism and by the immune system in defence against microbes, or produced as reactions to exogenous exposures such as cigarette smoking, imbalanced diet,

exhaustive exercise, environmental pollutants and food contaminants. Oxidative stress may modify important molecules in the body forming atherogenic, carcinogenic, diabetogenic and brain degenerating substances [36].

In this study a simple colorimetric assay of total antioxidant capacity was chosen to determine antioxidant stress. This assay which was chosen primarily for practical reasons, measures combined reducing capacity. Although useful for testing the antioxidant strength of a food, it has limited value for assessing antioxidant status of plasma. The sensitivity of such assays is too low to detect the very small changes resulting after antioxidant supplementation (above the background of albumin and uric acid reacting in the assay). More useful markers of antioxidant stress may include oxidative DNA damage (e.g. 8-oxo-7,8-dihydro-2'-deoxyguanosine) or measures of lipid oxidation (e.g. isoprostanes) [37].

Despite the limitations of the antioxidant status method used in the present study, the results demonstrate poor antioxidant status amongst the subjects. Furthermore, it is interesting to note that the antioxidant status of most subjects appeared to improve while being fed in the field and this may be good news for the CRP menus in terms of antioxidant content.

4.2.4 Protein catabolism

The visceral proteins fibronectin and IGF-1 are both sensitive to dietary restriction, in particular energy restriction [unpublished review, DNRC]. In this study IGF-1 was shown to be the single best marker of dietary restriction (Table 17) predicting 61% of the variation in energy intake. A linear model incorporating IGF-1 and sIgA:Alb predicted 69% of the variation in protein intake. Figure 8 illustrates the sensitivity of the plasma proteins IGF-1 and fibronectin to dietary restriction. Both these proteins were found to be negatively associated with macronutrient and energy intake (Table 17). The decline in plasma IGF-1 indicates the severity of the energy deficient diet for the subjects receiving the CRP menus and indicates that these subjects were in a catabolic state resulting in the loss of protein from body stores.

Fortunately this protein loss did not appear to extend to the loss of muscle as evidenced by normal urinary excretion of methylhistidines (Table 10) and no change in physical fitness (Table 5). Furthermore the concentration of the sensitive marker of acute phase response, plasma C-reactive protein, did not change for any subjects over the course of the training exercise and average plasma concentrations of IL6 significantly decreased amongst subjects in the two CRP groups. Intense exercise coupled with severe dietary restriction might have been expected to cause damage to skeletal muscle and thereby induce an acute phase response, which would result in elevated CRP and IL6 concentrations [38].

4.2.5 Immune system suppression

Because the immune system is large and the turnover and death rate of its cells is high, the immune system requires substantial amounts of nutrients. Nearly all aspects of the immune system can be impaired by nutritional deficiencies, and not just protein deficiency, but micronutrients as well. In general, cell-mediated and nonspecific immunity are more sensitive to nutritional status than humoral immunity [39]. T cell functions are more sensitive than B cells to most nutrient deficiencies. Thus a few specific immune tests such as lymphocyte mitogen assays, delayed cutaneous hypersensitivity and measurement of the cytokines, which modulate T-lymphocyte activity, can be useful indicators of nutritional status. The present study used DTH and the plasma cytokines, IL2, IL2r and IL6 as markers of cell-mediated immune function.

The CRP groups demonstrated an immune system suppression when compared with the Fresh group as evidenced by significant decrease in plasma concentrations of IL2 and IL6. However, there was no change in cell-mediated immunity as determined by the DTH test. The Fresh group, which experienced an increase in plasma IL2r concentration, demonstrated an immune system stimulation. The cytokines are very sensitive markers of immune function and the fact that there were no significant changes in the DTH test indicates that the immune suppression was measurable but not severe.

The saliva measurement IgA:Alb was used as a marker of humoral immune function. IgA represents only 15 to 20% of the serum immunoglobulin pool but it is the most abundant immunoglobulin in secretions, such as saliva, tears, colostrum and bronchial/intestinal/genitourinary secretions. Secretory IgA forms the first line of mucosal defence by providing specific antibodies in response to pathogens (bacteria and viruses), a transport mechanism for elimination of pathogens in the submucosa and an exclusion barrier at the mucosal surface to prevent pathogen entry. The lack of non-specific secretory IgA at mucosal surfaces or an inability to produce specific IgA antibodies results in an increased risk of infection [40].

One of the important findings in the present study was the significant decrease in sIgA:Alb experienced by each of the treatment groups. There was a significant dietary treatment effect with the CRP groups having lower sIgA:Alb than the Fresh group. The average sIgA:Alb recorded by each subject over the testing period correlated positively with their energy and macronutrient intake. The protein consumption of the subjects partially explained their sIgA:Alb value ($r^2 = 0.23$).

Overtraining, which is associated with an increased incidence of upper-respiratory tract infection amongst triathletes and elite swimmers, has been associated with decreased salivary IgA concentration [41]. During Exercise Northern Awakening the subjects in the study made 11 visits to the medical aide post for treatment of upper-respiratory tract infections. Unfortunately there was insufficient data to be able to correlate medical visits with the biochemical measures reported here.

Chronic stress has also been shown to result in decreased secretion of IgA into the saliva. Furthermore, the possibility that the relation between stress and secretory IgA may be moderated by personality characteristics or mediated by psychological distress has been supported by some studies [40]. The negative association between the mood factor, tension-anxiety and sIgA approached significance and the perceived loss of vigour by subjects was clearly associated with decreased levels of sIgA:Alb. Although the present study provides some evidence for the negative impact of stress upon immune function, much more research is required in this area.

4.2.6 Changes in mood and cognition

Despite the sleep and food deprivation experienced by most of the subjects, there was no change in the reported measures of cognition. There were some changes in mood. Increases in the mood factors fatigue and confusion and a decrease in vigour were recorded over the testing period. This is not surprising given the combined effects of high-level physical exercise, decreased food consumption and sleep deprivation. Of greater relevance is that subjects eating CRP reported greater levels of fatigue and negative emotions than the Fresh group. In particular they felt more confused and depressed. The effect of diet on mood requires further investigation.

As with the metabolic, cognitive and fitness measures mentioned above, the mood ratings provide further evidence of adaption to the restricted CRP diet. Results for vigour, fatigue and depression are illustrative. After the first 6 days the vigour ratings tend to stabilise and show no difference between the Full CRP and Half CRP groups. Both fatigue and depression gradually increase over the course of the exercise, again with no difference between the two CRP groups.

4.3 Experimental design

As explained in Methods and Materials it was not practical to allocate dietary treatment in a random fashion. This has implications for testing for differences in dietary effects. There may be unidentified sources of variation among the groups that are not present among the members within each group. Hence the residual variance as measured among individuals within groups could underestimate the level of chance variation among groups. The consequence is that tests of differences in responses among the different dietary groups may have given false evidence of treatment effects.

The likelihood of false treatment effects is greater if there are identifiable differences in the composition of the groups or in the way in which the groups operate during the training exercise. To this end, a (RAAF) member of the research team made a detailed observation of the activities conducted by the groups and this report reassured the authors that the groups conducted similar tasks and operated with similar work intensity. Further evidence of the homogeneity of the groups, included the estimation of energy expenditure in the two CRP groups and the Actigraph data (sleep quality

and activity). The differences in these measurements between the groups were not significant. Furthermore, to compensate for the experimental design problem, all the analyses were based upon the use of baseline-adjusted responses.

5. CONCLUSIONS

1. The menu, which was designed by use of REAP™, was found to maintain the nutrition status of subjects in the Fresh group.
2. Freshly prepared foods were better consumed than CRP foods. A high rate of ration item discards by ADGs being fed CRP as their sole nutrition resulted in these subjects eating insufficient food for their energy and carbohydrate needs.
3. The effects of inadequate sleep and high physical demand under the hot humid conditions of this training exercise coupled with food deprivation were not sufficient in the medium-term (12 days) to result in a measurable loss of physical fitness or cognition. Furthermore there was metabolic and psychological evidence that subjects eating CRP were able to adapt to a restricted dietary intake, because there were no differences in metabolic, physical or psychological measures between the two CRP groups.
4. Restricted food consumption by the subjects in the CRP groups resulted in mild symptoms of weight loss, suppressed immune function, loss of visceral protein, increased fatigue, loss of vigour, and increased feelings of confusion. Carbohydrate intake was negatively associated with run times during the fitness testing.
5. Vitamin status of the ADGs as determined by measurement of homocysteine, vitamin K and total antioxidant capacity was poor before Exercise Northern Awakening and tended to be worse on completion of the exercise.
6. There was some evidence that smoking suppressed immune system function and exacerbated weight loss. These results were not conclusive.
7. Serum IGF-1 and salivary sIgA:Alb were sensitive biomarkers of dietary energy deficit.

6. Recommendations

1. Future CRP acceptability studies should focus on understanding the reasons why soldiers discard up to one quarter of the items in the CRP pack.
2. Further research is required to evaluate the potential of alternate CRP items or supplements, which may reverse adverse metabolic and psychological effects.
3. The finding of poor micronutrient status amongst the ADGs lends support to the need for a comprehensive nutrition education program for ADF personnel. The findings are important and require further investigation, because poor vitamin K, folic acid and antioxidants status are indicators of increased cardio-vascular disease risk.
4. Although not conclusive, cigarette smoking was implicated in decreased military performance. A detailed study of the effects of smoking during training exercises may be warranted.
5. The present study was of medium duration (12 days). A similar CRP study over 30 days is required to determine the effect of ration pack feeding during sustained operations. Such a study should also monitor the subjects during the recovery phase after completion of the training exercise.

7. Acknowledgements

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Appendix A: Consent and Information Forms

CONSENT FORM

VITAMIN STATUS OF SOLDIERS: COMBAT RATION PACK STUDY

Task No: DST 97/126

I, give my consent to participate in the project mentioned above on the following basis:

- I have had explained to me the aim of this research project, how it will be conducted and my role in it. I am happy to participate.

I understand that I have agreed to have my mental, physical and nutritional status measured. This will involve completion of psychological, medical and dietary questionnaires, the donation of 2 fasting blood samples (20 mL each), several urine samples and completion of physical fitness tests. I understand that the protocol of taking and handling my blood samples will conform to conventional medical practice and that the risk to myself of a deleterious outcome will be no higher than for a routine medical examination.

I understand that blood tests will include measures of fat metabolism (β -hydroxybutyrate), vitamin status (folic acid, thiamin, riboflavin, total antioxidants, vitamin K, vitamin B6, homocysteine, F2 α isoprostane), protein status (fibrinogen, insulin-like growth factor), and immune function (interleukin). Urine tests will measure hydration status (osmolality and sodium), labelled water components and muscle protein turn-over (3-methylhistidine, 1-methylhistidine, creatinine).

- I understand that I am participating in this project in a voluntary capacity and can withdraw at any time without detriment to my career and without compromise to my medical care.
- I am co-operating in this project on condition that:
 - no other tests will be performed without my consent;
 - the information I provide will be kept confidential;
 - the information will be used only for this project;
 - the research results will be made available to me at my request and
 - any published reports of this project will preserve my anonymity.

I have been given a copy of the information sheet and this form, signed by me and by the principal researcher, Dr Christine Booth.

.....
SUBJECT	DATE
.....
CHRISTINE BOOTH	DATE

INFORMATION SHEET

VITAMIN STATUS OF SOLDIERS

Task No: DST 97/126

There have been few field evaluations of combat rations, even internationally. In fact Australian combat rations haven't been put-to-the-test since the 1960's.

Soldiers participating in a 34-day American study experienced weight loss and a 23% decrease in lifting strength and in another American study a dramatic suppression of immune function coinciding with increased rate of infection was reported. Military effectiveness will be compromised when effects such as these are experienced.

This is an important study. Nutrition is essential for soldiers to be able to maintain optimal physical performance in the field. Studies like this provide data on the operational effectiveness of combat rations and enable scientists to continually improve ration pack design.

1. You will be asked to complete some short questionnaires, which request information about what you eat and your medical fitness and which assess your mental ability and mood state. These forms will be handed out at various times before, during and after the field study¹.
2. Before commencing the field study and again on completion of the study you will be asked to perform a fitness test and to donate a small fasting blood sample (20 mL). See below for a detailed description of the fitness test. The fitness test may require up to 5 finger prick blood samples. If you feel that you cannot continue with any test, you will be able to stop immediately. Because some soldiers can push themselves beyond exhaustion, we will also stop your participation if signs of heat exhaustion or stress are detected from you.
3. In order to determine your energy expenditure, some of you will be given either a "labelled" or plain water sample. Naturally occurring water consists of Hydrogen and Oxygen atoms with a range of slightly differing atomic weights (or isotopes). The "labelled" water has been specially purified to contain specific stable isotopes. Stable isotopes are NOT radioactive. This is a commonly used procedure for measuring energy expenditure in adults, children and newborn babies.
4. You will be asked to provide single urine samples on several days before, during and after the study. These samples will be used to determine muscle turnover (3-methylhistidine, 1-methylhistidine, creatinine), energy expenditure (labelled water components) and hydration status (osmolality, sodium).

Blood tests will include measures of fat metabolism (β -hydroxybutyrate), vitamin status (folic acid, thiamin, riboflavin, total antioxidants, vitamin K, vitamin B6, homocysteine, F2 α isoprostane), protein status (fibronectin, insulin-like growth factor), and immune function (interleukin). Urine tests will measure hydration status (osmolality and sodium)

¹ The scheduled course/exercise name will be included on the form. A timetable for the course, which includes the timing of questionnaires, fitness testing, blood and urine collections, will be distributed.

and muscle protein turn-over (3-methylhistidine) and labelled water components (energy expenditure determination).

The risk to you of deleterious outcomes during and/or after the study will be no higher than for routine medical examination. The blood collector will place a tourniquet around your upper arm then draw a blood sample from the inside of your arm into a syringe. Apart from a small prick when the needle pierces the skin, little discomfort is experienced by most people. However, if the procedure makes you feel faint, you should remain sitting and place your head between your knees. By applying pressure on the puncture site after the needle is withdrawn, bruising should be prevented. The use of sterile technique by the blood collector will prevent the risk of infection. Blood sampling by fingerprick will be performed in a controlled laboratory situation with appropriate infection control. Apart from some local pain (sting) and possible bruising, risk of side-effects from fingerprick blood sampling is minimal.

You will be asked to consume no food for approximately 12 hours before the 'fasting' blood sample is collected. Usually this means not eating food from 2100 hours the night before donating the blood sample. The combat ration study will be conducted whilst you are 'on duty'. A trained data collector will assist you in the completion of the survey forms and a qualified dietitian/nutritionist will assist you to complete the food frequency questionnaires. After analysis of the data you will be provided with feedback on this dietary information as well as blood chemistry status.

Your participation in this research is voluntary and refusal to be involved will entail no detriment to your career. You may discontinue at any time without compromise to your medical care. The information collected will be kept confidential and nothing will be published which will identify individual participants. The information will only be used for the stated aims, above.

Should you have any complaints or concerns about the manner in which this research is conducted, please do not hesitate to contact the chief investigator:

Dr Christine Booth, DSTO-Defence Nutrition Research Centre
76 George St, Scottsdale TAS 7620
Ph : 03 6352 2033, Fax: 03 6352 3044; Email: christine.booth@dsto.defence.gov.au

OR you may contact the Australian Defence Medical Ethics Committee:

Executive Secretary, Australian Defence Medical Ethics Committee
Office of Surgeon General Australian Defence Force
CP4-6-45, Campbell Park Offices
CANBERRA ACT 2601
Ph : 06-2663921 DNATS 8 66 3921, Fax: 06 2664982 DNATS 8 66 4982

FITNESS TEST:

- (i) Maximal oxygen uptake (VO_{2max}) will be measured on a treadmill using open circuit spirometry. VO_{2max} is regarded as the best all-round measure of the capacity to

engage in sustained physical work. The aerobic test is a standard VO_2 max test as routinely performed for testing athletes. The test involves the following procedure:

Subjects will be fitted with an external 'Polar' heart rate monitor to monitor heart rate throughout the test. Subjects will first warm up by walking on the treadmill for 5 minutes at 5-6 km/h. The VO_2 Max test will begin at a light run on the treadmill with the speed being increased at set time intervals. Once a maximum speed of 18 km/h for males has been reached, if the subject is happy to continue, the incline of the treadmill will then be increased. The incline will continue to be increased at 1 min intervals until the subject reaches exhaustion. Throughout the duration of the test the subject will breathe into a mouthpiece held steady on the head by a head piece. A nose clip will ensure mouth breathing. The subject is free to hit the emergency stop button on the treadmill at any time during the test should they want to stop for any reason. After the test is completed the mouthpiece and nose clip will be removed and the subject will walk on the treadmill for approximately 5 min to warm down.

Blood lactate levels are monitored by finger prick samples. Two to five finger prick samples will be taken over the duration of the test. Under most circumstances this will be

two to three samples. The treadmill will be momentarily stopped for safety reasons while the blood is being sampled.

- (ii) Upper body strength will be assessed by conducting the upper body component of the Army's Basic Fitness Assessment. This involves a flexed arm hang which is continued until voluntary exhaustion.

Appendix B: Experimental CRP menu

B.1. Full CRP menu (15000 kJ menu)

Main Meal Items, select 2

Item	Wt	kJ	kcal	Prot	Fat	Carb
VEAL ITALIENNE	85	1510	360	31.0	11.0	36.0
BEEF, SAVOURY STEAK FINGERS, FD	75	1546	369	41.3	17.9	11.3
SPAGHETTI&MEATBALLS	225	1487	355	24.3	23.2	13.5
BEEF & BLACK BEAN SAUCE FD	75	1360	320	32.0	16.5	13.0
BEEF TERIYAKI FD	75	1490	315	35.0	14.5	13.0
BEEF MINCED WITH SPAGHETTI	500	1890	445	51.8	11.7	36.2
BEEF KAI SI MING	500	1925	450	41.7	14.1	43.5
BEEF & PASTA	500	1800	420	43.1	11.6	36.8
SAUSAGES TOMATO&ONION	500	1720	410	19.0	23.0	34.0
Average values:	282	1636	383	35.5	16	26.4
SD:	212	202	51	10.2	4.7	13.2

Vegetables - starch, select 1

Item	Wt	kJ	kcal	Prot	Fat	Carb
RICE, WHITE, FREEZE DRIED	55	835	200	4.0	0.3	47.2
CHICKEN INSTANT NOODLES	37	690	165	4.0	7.0	22.8
BAKED BEANS	225	947	226	9.3	1.3	46.4
POTATO&ONION, DRIED	50	797	190	5.8	3.1	36.4
BEEF INSTANT NOODLES	37	710	169	3.8	7.3	23.1
Average value:	80.8	796	190	5.4	3.8	35.2
SD:	81	104	25	2.3	3.2	12.0

Soups, select 1

Item	Wt	kJ	kcal	Prot	Fat	Carb
SOUP, FRENCH ONION, DRY(CALC)	10	114	27	1.7	0.0	5.1
TOMATO S&G BASE	10	127	30	0.6	0.2	6.7
BEEF S&G BASE	10	131	31	0.9	0.5	6.1
CHICKEN S&G BASE	10	135	32	0.9	0.9	5.4
PEA&HAM S&G BASE	10	141	34	1.0	0.4	6.9
Average:	10	127	30	1	0.4	5.8
SD:	0	10	2	0.4	0.3	0.8

Dairy, select 2

Item	Wt	kJ	kcal	Prot	Fat	Carb
CHEESE,CHEDDAR,PROCESSED	56	778	186	12.0	15.4	0.2
MILK,SWEET	85	1156	276	7.2	7.6	47.0
CONDENSED,WHOLE,TUBE						
Average:	70.5	967	231	9.6	12	23.6
SD:	20.5	267	64	3.4	5.5	33.1

Fruits, select 4

Item	Wt	kJ	kcal	Prot	Fat	Carb
Apricot fruit grains	15	200	48	0	0	10
Blackcurrant fruit grains	15	200	48	0	0	10
Mixed berry fruit grains	15	200	48	0	0	10
Orange fruit grains	15	200	48	0	0	10
Tropical fruit grains	15	200	48	0	0	10
JAM,APRICOT	26	272	65	0.1	0.0	16.8
JAM,PEACH	26	274	66	0.1	0.0	17.0
JAM,BLACKBERRY	26	275	66	0.1	0.0	17.1
JAM,RASBERRY	26	278	66	0.1	0.1	17.2
JAM,PLUM	26	285	68	0.1	0.0	17.7
PEACH,CAN-PEAR JUICE	140	238	57	0.8	0.0	13.0
PEAR,CAN-PEAR JUICE	140	252	60	0.7	0.0	14.8
TWO FRUITS,CANNED	140	472	113	0.6	0.1	28.6
weighted Average:	33.6	227	54	0.1	0	12
SD:	45.2	73	17	0.3	0	5

Sugar, select 4

Item	Wt	kJ	kcal	Prot	Fat	Carb
SUGAR,WHITE (4 PKT)	28	448	11	0.0	0.0	28.0
ERGO™ DRINK (= 2 choices)	47	720	170	0.0	0.0	45.0
SUGAR CONFECTIONERY,HARD TYPE	30	474	113	0.0	0.0	29.5
CHOCOLATE DRINK POWDER	20	317	76	1.3	0.7	16.8
Average:	25	400	71	0.3	0.2	24.0
SD:	4.5	74	13	0.6	0.4	5.8

Biscuits, select 2

Item	Wt	kJ	kcal	Prot	Fat	Carb
BISCUIT,SHORTBREAD	35	721	172	2.1	8.7	21.7
BISCUIT,JAM SANDWICH	45	806	192	2.0	8.0	29.1
BISCUIT,GINGERNUT	40	691	165	2.3	4.0	31.5
BISCUIT,ANZAC	35	676	161	2.2	8.4	19.9
CRISPBREAD,(2 pkts)	60	985	236	7.8	2.4	4.8
Average:	43	776	185	3.3	6.3	21.4
SD:	10.4	127	30	2.5	2.9	10.5

Muesli bar,select 2

Item	Wt	kJ	kcal	Prot	Fat	Carb
MUESLI BAR,TROP FRUIT	32	556	133	1.9	3.8	23.9
MUESLI BAR,THREE FRUITS	32	552	132	2.0	4.1	22.9
MUESLI BAR,FOREST FRUIT	32	496	118	1.5	4.7	17.9
MUESLI BAR,APRICOT&C'NUT	32	563	135	2.0	4.5	22.7
MUESLI BAR,ANZAC	32	627	150	3.1	7.2	18.0
Average:	32	559	134	2.1	4.9	21
SD:	0	47	11	0.6	1.4	3

Choc/energy bars, select 3

Item	Wt	kJ	kcal	Prot	Fat	Carb
CHOCOLATE,MILK	50	1075	257	4.2	13.7	31.0
CHOCOLATE,CANDY-COATED	60	1188	284	2.9	10.6	46.0
HOOAH!™ BAR	65	1000	235	4.2	18.0	30.0
Average:	58.3	1088	259	3.7	14	35.7
SD:	7.64	95	24	0.8	3.7	8.9

Beverage powders, select 1

Item	Wt	kJ	kcal	Prot	Fat	Carb
TROPICAL BEVERAGE POWDER	12	174	42	0.2	0.1	10.4
ORANGE BEVERAGE POWDER	12	174	41	0.2	0.2	10.3
LIME BEVERAGE POWDER	12	173	41	0.2	0.1	10.2
LEMON BEVERAGE POWDER	15	220	53	0.2	0.2	13.1
Average:	12.8	185	44	0.2	0.2	11
SD:	1.5	23	6	0.0	0	1

ITEM GROUP	NO selected	Wt	kJ	kcal	Prot	Fat	Carb
Main meal	2	290	1636	383	36	16	26.4
Starch	1	81	790	190	5.4	3.8	35.2
Soups	1	10	127	30	1	0.4	5.8
Dairy	2	71	967	231	9.6	12	23.6
Fruit	4	41	230	54.8	0.2	0	12.2
Sugar	4	25	400	71	0	0	24
Biscuits	2	43	776	185	3.3	6.3	21.4
Muesli bar	2	32	560	134	2.1	4.9	21
Energy/choc bar	3	58	1088	259	3.7	14	35.7
Beverage	1	13	185	44.2	0.2	0.2	11
Common items	1	70.3	370.6	88.5	6.9	2.1	11
Totals:		1484	15135	3897	126	127	500
Average % of energy:					15	31	53

Within each group, subjects can select the number of items prescribed to make up a 24 hour CRP.

B.2. Half CRP menu (7500 kJ menu)**Main Meal Items, select 2**

Item	Wt	kJ	kcal	Prot	Fat	Carb
SAUSAGES TOMATO&ONION	225	860	205	9.6	11.4	17.0
BAKED BEANS	225	947	226	9.3	1.3	46.4
VEGETABLES&SAUSAGES,CAN,HE ATED	225	770	185	8.8	9.5	16.0
BEEF KAI SI MING	225	960	226	20.9	7.1	21.8
TUNA MORNAY FD	50	970	231	17.5	11.0	17.5
BEEF TERIYAKI FD	50	992	236	26.0	11.0	9.8
BEEF & BLACK BEAN SAUCE FD	50	1020	239	23.8	12.5	9.7
LAMB CASSEROLE FD	50	1034	246	17.4	12.0	18.5
Average values:	138	944	224	16.7	9.5	19.6
SD:	94	88	20	6.8	3.7	11.6

All other Groups as for the 15000 kJ menu.

ITEM GROUP	NO selected	Wt	kJ	kcal	Prot	Fat	Carb
Main meal	2	138	944	224.0	16.7	9.5	19.6
Starch	1	81	790	190.0	5.4	3.8	35.2
Soups	0	10	127	30.0	1.0	0.4	5.8
Dairy	1	71	967	231.0	9.6	12.0	23.6
Fruit	2	41	230	54.8	0.2	0.0	12.2
Sugar	2	25	400	71.0	0.0	0.0	24
Biscuits	2	43	776	185.0	3.3	6.3	21.4
Muesli bar	1	32	560	134.0	2.1	4.9	21
Energy/choc bar	0	58	1088	259.0	3.7	14.0	35.7
Beverage	1	13	185	442.0	0.2	0.2	11
Common items	1	70	371	88.5	6.9	2.1	11
Totals:		761	7573	2155.1	64.4	54.6	256
Average % of energy:					14	27	54

Within each group, subjects can select the number of items prescribed to make up a 24 hour CRP.

B.3. Brew kit issued to Fresh group**3 Day Ration**

ITEM GROUP	NO selecte d	Wt	kJ	kcal	Prot	Fat	Carb
CHEESE,CHEDDAR,PROCESSED	1	56	778	186	12.0	15.4	0.2
MILK CONDENSED,TUBE	1	85	1156	276	7.2	7.6	47.0
Fruit grains 3 different flavours	6	15	200	48	0	0	10
SUGAR,WHITE (4 PKTs of 7g)	1	28	448	11	0.0	0.0	28.0
ERGO™ DRINK (= 2 choices)	1	47	720	170	0.0	0.0	45.0
SUGAR CONFECTIONERY,HARD TYPE	3	30	474	113	0.0	0.0	29.5
CRISPBREAD,(2 pkts = 1 choice)	1	60	985	236	7.8	2.4	4.8
BISCUIT choice	2	45	806	192	2.0	8.0	29.1
Energy/choc bar	3	58	1088	259.0	3.7	14.0	35.7
Beverage	1	13	185	442.0	0.2	0.2	11
Common items	1	70	371	88.5	6.9	2.1	11
Totals:		803	12140	3199	49	86	461
per day:		268	4047	1066	16	29	154

B.4. Supplementary items issued to each treatment group

Common items	packaged as one unit					
Item	Wt	kJ	kcal	Prot	Fat	Carb
VEGEMITE	17	100	24	4.2	0.2	1.4
SAUCE,TOMATO,COMMERCIAL	17	72	17	0.2	0.0	4.1
SAUCE,CHILLI	17	74	18	0.2	0.4	3.2
MUSTARD,DRY	2	45	11	0.6	0.8	0.3
CURRY POWDER	2.8	35	8	0.4	0.4	0.9
COFFEE POWDER,INSTANT	10.5	45	11	1.4	0.2	1.1
TEA BAG	4	0	0	0.0	0.1	0.0
CHEWING GUM						
SALT						
PEPPER						
TABASCO SAUCE						
MATCHES, WATERPROOF						
CAN OPENER						
SPOON, PLASTIC						
RUBBER BAND						
TOILET PAPER						
PAD SCOURING WITH SOAP						
BAG PLASTIC						
Total:	70.3	370.6	88.53	6.88	2.1	11

Appendix C: Fresh group menu (15 000 kJ)

SAMPLE MENUS FOR 15000 KJ (recipes and daily menus provided)

AA

Breakfast

READY-TO-EAT CEREAL 60.00 G (1 CUP OR 4 BISCUITS)
 MILK, LOW FAT, MODIFIED, FLUID 1.00 CUP (250 ML)
 FRUIT JUICE, COMMERCIAL 0.50 CUP (125 ML)
 BREAD, MIXED GRAIN, TOASTED 2.00 SLICE
 MARGARINE, DOMESTIC, UNSPEC TYPE 10.00 G (2 TEASPOONS)

HOT MEAL (FRENCH TOAST OR PAN CAKES OR EGG WITH BAKED BEANS/THIN
 SAUSAGE, TOMATO, AND BACON)

Tea/coffee

Lunch/dinner

COLD MEAT SALAD SERVED ON A PLATE OR AS SALAD ROLLS OR CUT
 SANDWICHES
 FRESH FRUIT
 MILK DRINK (REDUCED FAT) OR LOW-FAT YOGHURT

Tea/coffee

Dinner/lunch

BEEF OR VEAL OR LAMB 150.00 G
 PASTA, OR RICE OR POTATO 1 CUP/10 CHIPS/1 MEDIUM
 POTATO/0.5 CUP MASHED POTATO
 GREEN VEGETABLE (1 OR 2 SERVES) 0.5 CUP PER SERVE
 CARROT OR PUMPKIN OR CORN 0.50 CUP

BREAD, MIXED GRAIN (2 SLICE) WITH MARGARINE OR CAKE SLICE OR LOW-
 FAT MILK DESSERT

Tea/coffee

Field ration (supplied by DNRC)

Tea, coffee, chewing gum, matches, plastic spoon, can opener, toilet
 paper, beverage powder, condensed milk/cheese, fruit grains/canned
 fruit, sugar, chocolate drink powder/candy, biscuits/crisp bread,
 energy bar (chocolate/M&Ms/HoohAh!™ bar)

Appendix D: POMS and ESQ Questionnaires

EXERCISE NORTHERN AWAKING - ENVIRONMENTAL SYMPTOMS QUESTIONNAIRE

Reg NO:

Last Name:

Sample number:

Time (24 hours):

Date:

MARKING INSTRUCTIONS

Below is a list of symptoms.

Please read each one carefully;
then

• Use a No. 2 pencil only.

mark ONE circle under the answer to the right which best describes HOW YOU FEEL RIGHT NOW. If you are not experiencing the symptom, fill in the response labeled "NOT AT ALL." The numbers refer to these phrases:

0 = Not at all

3 = Moderately

1 = Slightly

4 = Quite a
bit

2 = Somewhat

5 =
Extremely

0 1 2 3 4 5

0 1 2 3 4 5

0 1 2 3 4 5

I feel lightheaded

0 1 2 3 4 5

I have a stomach ache

0 1 2 3 4 5

My ears were ringing

0 1 2 3 4 5

I have a nose bleed

0 1 2 3 4 5

I am shivering

0 1 2 3 4 5

My nose is stuffed up

0 1 2 3 4 5

I have sinus pressure

0 1 2 3 4 5

I have gas pain

0 1 2 3 4 5

I am sweating all over

0 1 2 3 4 5

I feel dizzy

0 1 2 3 4 5

I have diarrhea

0 1 2 3 4 5

I have a headache

0 1 2 3 4 5

I feel constipated

0 1 2 3 4 5

I have a muscle cramp

0 1 2 3 4 5

I have chest pressure

0 1 2 3 4 5

My vision is dim

0 1 2 3 4 5

I feel restless

0 1 2 3 4 5

My throat is sore

0 1 2 3 4 5

My coordination is off

0 1 2 3 4 5

I feel warm

0 1 2 3 4 5

I am coughing

0 1 2 3 4 5

I am short of breath

0 1 2 3 4 5

I feel faint

0 1 2 3 4 5

I have lost my appetite

0 1 2 3 4 5

I feel sleepy

0 1 2 3 4 5

I feel feverish

0 1 2 3 4 5

I feel sick

0 1 2 3 4 5

It is hard to breathe

0 1 2 3 4 5

My feet are sweaty

0 1 2 3 4 5

I feel hung over

0 1 2 3 4 5

My heart is beating fast

0 1 2 3 4 5

My nose is running

0 1 2 3 4 5

I am thirsty

0 1 2 3 4 5

My vision is blurry

0 1 2 3 4 5

My hands are cold

0 1 2 3 4 5

I feel tired

0 1 2 3 4 5

I have chest pain

0 1 2 3 4 5

My feet are cold

0 1 2 3 4 5

My heart is pounding

0 1 2 3 4 5

My hands are shaking

0 1 2 3 4 5

I feel nauseous

0 1 2 3 4 5

I feel wide awake

0 1 2 3 4 5

My mouth is dry

0 1 2 3 4 5

I feel chilly

0 1 2 3 4 5

My concentration is off

0 1 2 3 4 5

I am more forgetful than
usual

0 1 2 3 4 5

I need to urinate
MORE than usual

0 1 2 3 4 5

Parts of my body feel
numb

0 1 2 3 4 5

I have stomach cramps

0 1 2 3 4 5

My eyes are
irritated

0 1 2 3 4 5

I am worried or nervous

0 1 2 3 4 5

My muscles are tight

0 1 2 3 4 5

My skin
burns/itches

0 1 2 3 4 5

I am irritable

0 1 2 3 4 5

I feel weak

0 1 2 3 4 5

It hurts to breathe

0 1 2 3 4 5

I need to urinate LESS

0 1 2 3 4 5

My ears ache

0 1 2 3 4 5

My ears are blocked

0 1 2 3 4 5

I am bored

0 1 2 3 4 5

My back aches

0 1 2 3 4 5

I can't hear well

0 1 2 3 4 5

I feel depressed

0 1 2 3 4 5

I feel alert

0 1 2 3 4 5

I am hungry

0 1 2 3 4 5

My hands, arms or

0 1 2 3 4 5

I feel good

0 1 2 3 4 5

My legs and feet ache

0 1 2 3 4 5

shoulders ache

0	1 2 3 4 5 6 7 8 9	PLEASE DO NOT WRITE	0	1 2 3 4 5 6 7 8 9
0	1 2 3 4 5 6 7 8 9	IN THIS SECTION	0	1 2 3 4 5 6 7 8 9
Reg. 0	1 2 3 4 5 6 7 8 9		Sample 0	1 2 3 4 5 6 7 8 9
Number 0	1 2 3 4 5 6 7 8 9		Number 0	1 2 3 4 5 6 7 8 9
0	1 2 3 4 5 6 7 8 9 0 1 2	3 4 5 6 7 8 9	0	1 2 3 4 5 6 7 8 9
	Test			
0	1 2 3 4 5 6 7 8 9 0 1 2	3 4 5 6 7 8 9	0	1 2 3 4 5 6 7 8 9
0	1 2 3 4 5 6 7 8 9 0 1 2	3 4 5 6 7 8 9	0	1 2 3 4 5 6 7 8 9

PROFILE OF MOOD STATES

Last Name:

Subject Number:

Date:

Below is a list of words that describe feelings people have. Please read each one carefully; then mark ONE circle under the answer to the right which best describes **HOW YOU FEEL RIGHT NOW.**

The numbers refer to these phrases:

- 0 = Not at all
1 = A little
2 = Moderately
3 = Quite a bit
4 = Extremely

MARKING INSTRUCTIONS

- Use a No. 2 pencil only.
- Do not use ink, ballpoint, or felt tip pens.
- Make solid marks that fill the response completely.
- Erase cleanly any marks you wish to change.
- Make no stray marks on this form.

CORRECT:

INCORRECT:

Friendly	0 1 2 3 4	Unworthy	0 1 2 3 4	Desperate	0 1 2 3 4
Tense	0 1 2 3 4	Spiteful	0 1 2 3 4	Sluggish	0 1 2 3 4
Angry	0 1 2 3 4	Sympathetic	0 1 2 3 4	Rebellious	0 1 2 3 4
Worn out	0 1 2 3 4	Uneasy	0 1 2 3 4	Helpless	0 1 2 3 4
Unhappy	0 1 2 3 4	Restless	0 1 2 3 4	Weary	0 1 2 3 4
Clearheaded	0 1 2 3 4	Unable to concentrate	0 1 2 3 4	Bewildered	0 1 2 3 4
Lively	0 1 2 3 4	Fatigued	0 1 2 3 4	Alert	0 1 2 3 4
Confused	0 1 2 3 4	Helpful	0 1 2 3 4	Deceived	0 1 2 3 4
Sorry for things done	0 1 2 3 4	Annoyed	0 1 2 3 4	Furious	0 1 2 3 4
Shaky	0 1 2 3 4	Discouraged	0 1 2 3 4	Efficient	0 1 2 3 4
Listless	0 1 2 3 4	Resentful	0 1 2 3 4	Trusting	0 1 2 3 4
Peeved	0 1 2 3 4	Nervous	0 1 2 3 4	Full of pep	0 1 2 3 4
Considerate	0 1 2 3 4	Lonely	0 1 2 3 4	Bad-tempered	0 1 2 3 4
Sad	0 1 2 3 4	Miserable	0 1 2 3 4	Worthless	0 1 2 3 4
Active	0 1 2 3 4	Muddled	0 1 2 3 4	Forgetful	0 1 2 3 4
On edge	0 1 2 3 4	Cheerful	0 1 2 3 4	Carefree	0 1 2 3 4
Grouchy	0 1 2 3 4	Bitter	0 1 2 3 4	Terrified	0 1 2 3 4
Blue	0 1 2 3 4	Exhausted	0 1 2 3 4	Guilty	0 1 2 3 4
Energetic	0 1 2 3 4	Anxious	0 1 2 3 4	Vigorous	0 1 2 3 4
Panicky	0 1 2 3 4	Ready to fight*	0 1 2 3 4	Uncertain about things	0 1 2 3 4
Hopeless	0 1 2 3 4	Good natured	0 1 2 3 4	Bushed	0 1 2 3 4
Relaxed	0 1 2 3 4	Gloomy	0 1 2 3 4		

*This means you are ready to get in a physical fight

PLEASE DO NOT WRITE IN THIS BOX

MAKE SURE YOU HAVE
ANSWERED EVERY ITEM

0	1 2 3 4 5 6 7 8 9			
0	1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	1	2 3 4 5 6 7 8
Subject				
0	1 2 3 4 5 6 7 8 9	TEST	0 1 2 3 4 5 6 7 8 9	GROUP
Number				
0	1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9		

Appendix E: Acceptability of carbohydrate supplements

HOOAH! BAR™ & ERGO DRINK™ ACCEPTANCE QUESTIONNAIRE RESULTS MEMORANDUM by Phil Niro

The 31 volunteers from the RAAF Airfield Defence Wing (AFDW) Air Defence Ground staff (ADGs) participating in the Defence Science and Technology Organization's (DSTO) Combat Ration Pack (CRP) study during Exercise Northern Awakening (Ex NA) were given a short questionnaire which asked them to indicate which flavours of Hooah Bar and Ergo Drink they consumed during the study and to rate those flavours on a 9-point scale. A copy of the questionnaire distributed is included as D.4. It should be noted that the volunteers were allowed to request which ration items they wanted as part of their three day ration packs distributed by the DSTO Study Team (TT) and therefore had the option of choosing which item and flavour they wanted to consume.²

E.1. HooAh!™ Bar

Of the total population, just over half of the group (N=16; 52%) responded that they had consumed Hooah Bars during Ex NA. Over two-thirds of those (N=11, 69%) indicated that they had eaten all flavours of Hooah Bars available. Ninety-four percent (N=15) reported having had both the chocolate and peanut butter flavours, and 75% (N=12) reported having had the raspberry.

When rating the flavour of the Hooah Bars using the 9-point scale (1=Dislike Extremely, 5=Neither Like or Dislike, 9=Like Extremely), the peanut butter bar was the most favourably rated, receiving a 7.6 (SD=2.3), or "Like Very Much." The chocolate bar also received a positive rating of 6.8 (SD=2.4), or "Like

²Subjects were given CRP menu choices in the categories; main meal, starch vegetables, soups, dairy, fruits, sugar (~25 g CH₂O and 400kJ), biscuits, muesli bars, energy bars (~35 g CH₂O and 1100 kJ), and beverage powders. The Ergo drink was offered as 2 sugar choices (sugar, Ergo drink, hard candy or chocolate drink powder) and the Hooah was offered as an energy bar choice (milk chocolate bar, candy-coated chocolate or Hooah bar). Subjects in the full CRP group could choose up to 12 sugar choices and 9 energy bars per three-day ration period. Subjects in the light CRP group could select up to 6 sugar choices and no energy bars per three-day ration period. However, several subjects in this group requested Hooah bars and received the Hooah bar in exchange for 2 muesli bars (up to 3 bars for a three-day ration period). Subjects in the group receiving fresh foods also received a CRP supplement which included up to 3 Ergo drinks and no Hooah bars per three-day ration period.

Moderately.” The raspberry-flavoured bar was given the lowest rating, 4.1 (SD=3.4), or “Slightly Disliked.”

The following table shows the breakdown of ratings by ration groups. No members of the Fresh Ration group indicated having had tried the Hooah Bar.

	Full Ration (n=10)	Half Ration (n=6)
	0 ± sd	0 ± sd
Chocolate	6.7 ± 1.6	7.0 ± 3.5
Raspberry	3.8 ± 3.0	4.6 ± 4.5
Peanut Butter	8.0 ± 1.1	7.0 ± 3.5

When asked what flavours the volunteers would like to see as future Hooah Bars, the most common responses were caramel (N=6) and vanilla (N=3).

E.2. Ergo Drink™

The majority of individuals (N=29, 94%) indicated that they consumed the Ergo Drink during Ex NA; however, less than a quarter of those (N=6, 21%) reported having had all five flavours of Ergo Drink available. The most commonly consumed flavours were the tropical punch (N=19, 66%) and raspberry-cranberry (N=18, 62%). Fifty-nine percent (N=17) had the orange flavour, just over half (N=15 52%) had the lemon flavour, and just less than half (N=14, 48%) had the lemon-lime.

Using the 9-point scale (1=Dislike Extremely, 5=Neither Like or Dislike, 9=Like Extremely), all Ergo Drink flavours received less than favourable ratings of “Moderately” to “Slightly Disliked,” with the raspberry-cranberry flavoured Ergo Drink receiving the highest rating of 4.4 (SD=3.7). Both the orange and the tropical punch flavours were given ratings of 3.8 (SD=3.5 and 3.4, respectively). Lemon was rated 3.7 (SD=3.6) and lemon-lime was rated 3.6 (SD=3.7).

The ease of Ergo Drink preparation was considered easy, rating a 4.1 (SD=0.9) using a 5-point scale (1=Very Difficult, 3=Neither Easy or Difficult, 5=Very Easy).

The following table presents the breakdown of Ergo Drink ratings by ration group.

	Full Ration (n=8)	Half Ration (n=10)	Fresh Ration (n=11)
	0 ± sd	0 ± sd	0 ± sd
Lemon	6.1 ± 2.5	2.5 ± 3.5	2.9 ± 3.7
Orange	5.5 ± 3.5	2.5 ± 3.3	3.7 ± 3.4
Tropical Punch	4.6 ± 2.9	4.4 ± 3.4	2.6 ± 3.7
Lemon-Lime	4.6 ± 3.9	3.1 ± 4.0	3.4 ± 3.4
Raspberry-Cranberry	6.1 ± 2.9	3.3 ± 3.7	4.1 ± 4.1

As seen in the table, with the exception of the Full Ration group, the volunteers gave the Ergo Drink mostly less than favourable ratings for all flavours, from "Dislike Very Much" to "Slightly Dislike." The Full Ration group gave both the Lemon and Raspberry-Cranberry drinks positive ratings of 6.1, or "Like Slightly," and the remaining three flavours the neutral rating of "Neither Like or Dislike." These higher ratings may be indicative of the larger percentage of members of the Full Ration group (N=5, 63%) having had tried all five flavours of Ergo Drink compared to only one member of the Half Ration and no members of the Fresh Ration groups indicating they had tried all flavours.

The most commonly requested future flavours for the Ergo Drink were a raspberry only flavour (N=4) and black currant (N=3).

All of the volunteers who had tried both the Hooah Bar and the Ergo Drink indicated that they preferred the Hooah Bar (N=14, 45%). Slightly more than half the group (N=17, 55%) reported having had not tried both items.

E.3. Focus Group Discussion and Comments/Suggestions

The study participants were asked to offer any comments or suggestions for improvements or changes to the Hooah Bar or Ergo Drink. Additionally, several volunteers were asked to take part in an informal focus group and were asked additional questions pertaining to the Soldier Systems Command (SSCOM) items. A list of the questions asked in these focus groups is included as D.5.

Overall, the HooAh!™ Bar received favourable comments and was well liked by the ADGs. One volunteer responded, "They are [expletive deleted] excellent!" Others described the bars as "handy; very nice" and the "HooAh!™ are great!" Focus group members described eating the bar when on patrol due to the ease of the item fitting in their chest pockets in much the same way as beef jerky sticks. They also described eating the bars before physical tasks, as a snack or dessert, and even as a main meal when time constraints prevented them from being able to sit down and consume the items from their ration packs. There were also a few requests for a "chocolate HooAh!™ that's not so rich."

An issue that arose during the course of the field exercise was the potential reflectiveness of the Hooah Bar packaging, both the outside label and inner white wrapper. Although most members of the focus group indicated that this was not a serious tactical problem, they did comment that the white inner package was visible at night, especially when standing within five metres (approximately 15 feet). This led to the suggestion that only one layer of packaging be used instead of the current two. Or, if two packages must be used, to change the colour of the inner wrapper to a more tactical one such as green or brown. Further comments offered supporting the elimination of one layer of packaging were the generation of less noise when opening and less waste produced after consuming. Other solutions to the reflectiveness issue included the peeling off of the outside label so that "it doesn't glow."

Although the ADGs gave the Ergo™ Drink the negative flavour ratings above, a potential explanation for this in light of their continued requests and use of the SSCOM item may be directly related to the preparation directions on the back label. These directions call for "[mixing] the contents of one package in 12 fluid ounces of water." Australia utilizes the metric system and this direction caused confusion, especially the first time the volunteers attempted to mix the Ergo Drink powder. This knowledge became known during the course of the study and may account for the multiple comments and suggestions that the Ergo™ Drinks needed more flavour. The volunteers reported that initially they were mixing one packet of powder in 500ml to 1 litre of water. This is approximately double to triple the 355ml required.

Others reported initially using less water than needed and that this sometimes caused the powder to not completely dissolve, producing "a jelly sludge" or a "claggy" (thick, gooey paste) substance at the bottom of their canteen cups. This did not discourage them from continuing to request the Ergo™ Drink, however. Since the majority of volunteers were mixing the Ergo™ Drink in their water bottle/canteens, which hold a volume of approximately 1 litre, they commented that they used a process of "hit or miss" on subsequent attempts to mix the drink before settling on two packets of Ergo™ Drink powder to their 1 litre water bottles.

E.4. Hooah™ bar & Ergo drink™ acceptance questionnaire

Reg No. _____ Last Name: _____ Sample No. _____



1. Please indicate which flavour(s) of Hooah™ Bar you consumed during this exercise. Circle all that apply.

- | | |
|-------------------|-------------------------------------|
| (a) Chocolate | (d) Consumed all flavours |
| (b) Raspberry | (e) Did not consume any Hooah™ Bars |
| (c) Peanut Butter | |

2. Please rate the flavour(s) of Hooah™ Bar you consumed during this exercise using the following scale:

	Dislike Extremely	Dislike Very Much	Moderately Dislike	Slightly Dislike	Neither Like or Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely		
	1	2	3	4	5	6	7	8	9		
Chocolate		1	2	3	4	5	6	7	8	9	N/A
Raspberry		1	2	3	4	5	6	7	8	9	N/A
Peanut Butter		1	2	3	4	5	6	7	8	9	N/A

3. What flavour(s) would you like to see as HooAh!™ Bars?

4. Please indicate which flavour(s) of Ergo Drink™ you consumed during this exercise. Circle all that apply.

- | | |
|--------------------|-------------------------------------|
| (a) Lemon | (e) Raspberry-Cranberry |
| (b) Orange | (f) Consumed all flavours |
| (c) Tropical Punch | (g) Did not consume any Ergo Drink™ |
| (d) Lemon-Lime | |

5. Please rate the flavour(s) of Ergo Drink™ you consumed during this exercise using the following scale:

Dislike	Dislike	Moderately		Slightly		Neither		Like		Like		Like		Like	
Extremely	Very	Much	Dislike	Dislike	Dislike	Like	or	Dislike	Slightly	Moderately	Very	Much	Extremely	Extremely	Extremely
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Lemon			1	2	3	4	5	6	7	8	9	10	11	12	N/A
Orange			1	2	3	4	5	6	7	8	9	10	11	12	N/A
Tropical Punch			1	2	3	4	5	6	7	8	9	10	11	12	N/A
Lemon-Lime			1	2	3	4	5	6	7	8	9	10	11	12	N/A
Raspberry-Cranberry			1	2	3	4	5	6	7	8	9	10	11	12	N/A

6. What flavour(s) would you like to see as Ergo Drink™?

7. Please rate the ease of Ergo Drink preparation using the following scale:

Very Difficult		Neither Difficult or Easy		Very Easy
1	2	3	4	5

8. Do you have any suggestions for improvements or changes to the Hooah™ Bar or Ergo Drink™?

9. If you have consumed both the Hooah™ Bar **and** the Ergo Drink™, which item do you prefer?

(a) HooAh!™ Bar

(b) Ergo Drink™

(c) Did not consume both items

E.5. Hooah™ bar & Ergo drink™ acceptance focus group questions

1. How did you prepare the Ergo™ Drink? Was it in: a) canteen cup; 2) canteen/water bottle; 3) other?

1a. Did the instructions on the back (ref: 12 oz. of water) confuse/cause problems?

1b. What volume of water did you use to mix the drink?

1c. Did the Ergo™ Drink completely dissolve in the water? Yes No
Didn't Notice

1d. If not, how would you describe what did not dissolve?

1e. If not, did this happen every time you tried to make the Ergo™ drink?

1g. If so, did this deter/discourage you from further using the Ergo™ Drink?

2. When did you eat the HooAh!™ Bar? Was it when you ate your rations, or was it when you were on patrol?

2a. If being eaten at on patrol or maneuvers at night, was the HooAh!™ Bar label noticeable? Was it reflective?

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19. ABSTRACT The effect of combat ration pack (CRP) feeding on military performance in a tropical environment was assessed. Three groups received different diets: freshly prepared foods (Fresh group), a Full CRP and a Half CRP during the conduct of a routine training exercise over 12 days. Physical, cognition, immune and nutritional status were recorded. Freshly prepared foods were better consumed than CRP foods. A high rate of CRP item discards resulted in subjects eating insufficient food for their energy and carbohydrate requirements and hence significant weight loss, protein catabolism and immune suppression were observed for the two CRP groups. All subjects experienced poor sleep quality with no effect of dietary treatment. Subjects eating CRP reported greater levels of fatigue and negative emotions than the Fresh group. All subjects had poor folate and vitamin K status, which tended to become worse during the exercise period. Subjects drank insufficient water to prevent dehydration and a high rate of cigarette smoking contributed to poor antioxidant status. Despite these negative effects, cognition and physical fitness were maintained over the course of the exercise.					

